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MICROSCOPICAL SECTION-CUTTING

Dr MARSH

MICROSCOPICAL SECTION-CUTTING

MICROSCOPICAL
SECTION-CUTTING

A PRACTICAL GUIDE

TO THE

PREPARATION AND MOUNTING OF SECTIONS FOR THE MICROSCOPE

SPECIAL PROMINENCE BEING GIVEN TO THE SUBJECT OF ANIMAL SECTIONS

BY

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SECOND EDITION

WITH SEVENTEEN ILLUSTRATIONS

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PREFACE TO SECOND EDITION.

THAT a large edition of a book devoted solely to the consideration of a SPECIAL DEPARTMENT of a *special subject* should have been exhausted in the short space of three years, is a fact that cannot fail to be gratifying to the author, showing as it does that, for a work of this description, there existed a real demand. The book would have been out of print much earlier but for the appearance in America—within three months after the publication of the work here—of a piratical *reprint*, which of necessity effectually stopped the sale of the English edition in that country.

In the present edition many new processes have been described, and several additional illustrations inserted, which, it is hoped, will greatly increase the usefulness of the book.

ST. HELENS,

March, 1882.

PREFACE TO THE FIRST EDITION.

If we glance at any of the various magazines devoted either wholly or in part to the subject of microscopy, we shall hardly fail to be struck with the numerous *Queries* relating to SECTION-CUTTING which are to be found in its pages. A simple explanation of this wide-spread want is afforded by the fact that the use of the microscope has at the present day extended to (and is still rapidly spreading amongst) vast numbers of students, who, in many instances, possess neither the leisure nor the means to refer for information to large and extensive text books. Moreover, were they actually to consult such works, they would practically fail to obtain the information of which they are in need, for the coveted instruction is to be found in those treatises only in a scattered and fragmentary form—no work, so far as we are

aware, treating of the subject in anything like a detailed manner. To fill this *vacuum* in the literature of microscopy the present manualette has been prepared. Little claim is made to originality, yet the book is by no means a mere compilation, but the outcome of long and extensive personal experience in the cutting and mounting of microscopical sections. Every process described has been put to the test of actual trial, so that its worth may confidently be depended upon. Many of the little points insisted upon in the ensuing pages will doubtless, to the practised microscopist, appear trivial or superfluous; but a vivid recollection of our own early failures and disappointments assures us that it is just these very *minutiæ* of detail which will be found most serviceable in directing and sustaining the faltering footsteps of the tyro.

St. Helens,

September, 1878.

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MICROSCOPICAL SECTION-CUTTING.

PART I.

On Preparing Tissues for Section.

1. *Introduction.*—Many of the most interesting objects with which the microscopist has to deal, cannot be made to reveal their beauty or minute structure until they have been cut into slices, or *sections*, of such a degree of thinness as to render them transparent, and thus permit of their examination by transmitted light, with objectives of varying power. Unfortunately, however, very few of the objects of this class are, in their natural condition, in a suitable state to be submitted to this method of procedure. Some are of such a soft and yielding nature, that any attempt to cut them is an utter failure, for in place of a perfect section being obtained, nothing remains

upon the knife but a mass of diffluent pulp; others, again, are of such density as to resist the action of any cutting instrument.

It is evident, therefore, that nothing can be done with such refractory materials until, by subjection to appropriate methods of preparation, they shall have been reduced to such a consistence as to render them suitable for cutting. How this is to be accomplished, will depend entirely upon the physical and chemical nature of the substance to be operated upon. As the various objects differ so widely from each other in these respects, so must the methods of preparation suitable to each also vary. It is clear, therefore, that no general directions for attaining this end can be given, which would be of any practical value. It is possible, however, and very convenient, to arrange the various objects into groups or classes, to the treatment of each of which certain general rules are applicable; but there will still remain a comparatively numerous series of objects whose individual peculiarities of structure will demand for them correspondingly special methods of preparation. When such objects come to be spoken of, the particular treatment most suitable to each will also be noticed.

2. *Preparation of Vegetable Tissues.*—In the

case of vegetable tissues, not only do we, as a rule, find their texture of too great density to be readily cut in their natural condition, but they also contain much resinous and starchy matter, of which it is highly desirable to get rid. In order to do this we first cut the substance (say, a stem, or root) into small pieces, which are to be placed in water for three or four days, by which time all the soluble gummy matters will have disappeared. The pieces are now transferred to a wide-necked bottle, containing methylated spirit, which, in the course of a few days, will dissolve out all the resin, etc. Many kinds of woody tissue are by these processes reduced to a fit condition for immediate cutting. Others, however, are so hard as to render it necessary to give them another soaking for some hours in water, to bring them to a sufficient degree of softness to cut easily. If the wood (as in some few refractory cases will happen) be still too hard for section, a short immersion in warm, or, if necessary, in boiling water, will not fail effectually to soften it. The treatment of such members of the vegetable division as require peculiar methods, will be found described in future pages.

3. *Preparation of Animal Tissues.—Animal* tissues differ from one another so greatly, both in consistence and chemical composition, as

well as in their degree of natural hardness, that no general rules can be given which would be applicable to the preparation of the whole class. Such as are of any considerable degree of hardness, as horn and kindred structures, must be treated much in the same manner as the denser varieties of wood—viz., by more or less prolonged immersion in water: cold, hot, or boiling. Those which are of extreme hardness—as bone and teeth—can be cut only by following certain special methods, full details of which will be found in the Sixth Part of this book. Many, and indeed the vast majority of animal tissues, offer a direct contrast, in point of hardness, to those we have just been considering. All the internal organs of the body are, when freshly removed, of much too soft a nature to permit, when in their unprepared condition, of easy or perfect cutting. It is upon bringing them to that critical degree of hardness, which is often so difficult to attain, that the chief secret of successful section-cutting depends; for, unless the hardening process has been carried up to, but not beyond, a given point, which varies with different tissues, the operator, however dexterous, will fail to obtain satisfactory sections. For if the hardening has fallen short of this critical point, he is to some extent in the same position as

if he were dealing with unhardened tissues; whilst if this point has been exceeded, the tissue will have become so brittle as to crumble before the knife.

For the purpose of hardening animal tissues the student has at his command two principal agents, namely, alcohol and chromic acid, each of which possesses advantages of its own, but the use of each of which is also attended by its own inconveniences. Thus, by the use of alcohol there is very much less risk of over-hardening the specimen than if chromic acid had been employed. Alcohol, however, though a capital indurating agent in some instances, does not answer so well in many others. Chromic acid is, therefore, to be preferred for general use. It is, however, a very delicate agent to manage, for, unless the greatest care be taken, it is exceedingly likely to over-harden tissues submitted to its action, and when this happens the specimen becomes utterly useless for cutting, as there is no known means of removing the extreme brittleness which it has acquired. By taking the precautions now to be given, this over-hardening may generally be avoided. Let us harden a portion of some viscus, say, the kidney, for instance. Suppose we cut from the organ five or six small pieces (from half to three-quarters of an inch square,

not larger); these must be placed in a mixture of equal parts of methylated spirit and water for three days, at the end of which period they may be transferred to a solution of chromic acid, made by dissolving twenty grains of the pure acid in sixteen ounces of distilled water. The solution should be kept in a wide-necked bottle furnished with a glass stopper. At the expiration of twenty-four hours, pour off the solution and replace it by fresh, and repeat this operation at the end of the first week. At the end of another week, carefully examine the immersed tissues, and, by means of a sharp razor, see if they have acquired the necessary degree of hardness to allow of a section of *moderate* thinness being made. If so, remove the pieces and put them into a stoppered bottle containing from six to eight ounces of methylated spirit. If, however, the hardening be found not to be sufficiently advanced, the chromic acid solution is to be poured off, and again replaced by fresh. It will now be necessary to examine the tissues at intervals of about two days, until they are found to be sufficiently hard, when they must be transferred to the spirit. Under no circumstances, however, should they be permitted to remain in the chromic acid longer than the end of the third week, and though they should at this

time appear not to have undergone sufficient induration, yet it will be advisable to transfer them to the methylated spirit, which, in a short time, will *safely* complete the process of hardening, without any risk being run of the tissue becoming ruinously brittle. Another advantage of removing tissues from the chromic acid solution at an early period is this, that the shorter the time tissues are acted upon by chromic acid, so much the more readily will they subsequently take the carmine stain, about to be described hereafter. It will be noticed that when the specimens have been transferred to the spirit, the latter will, in a day or two, become of a deep yellow colour, whilst a thick flocculent deposit falls to the bottom of the bottle. The tissues should then be removed, the bottle emptied and well washed, and, being refilled with clean spirit, the preparations are again to be transferred into it. This may occasionally be repeated until the spirit becomes, and remains, perfectly bright and clear. The specimens are then ready for section.

4. *Special Methods of Hardening.*—The brain, spinal cord, liver, and several other organs, etc., require special methods of hardening, details of which will be found in the paragraph devoted to each. In the case of *injected* preparations, the best plan is to harden them in

alcohol from the outset, beginning with weak spirit and gradually increasing its strength as the hardening proceeds. When the object has been injected with Prussian blue, a few drops of hydrochloric acid should be added to the alcohol to fix the colour.

It may here be observed that specimens of *morbid tissues* require, as a general rule, a shorter immersion in chromic acid solution than healthy tissues do. A very small degree of over-hardening speedily renders them brittle and useless. They should, therefore, be removed from the acid medium at the end of ten days or a fortnight, and their further hardening be carried on by means of alcohol.

PART II.

On Cutting the Section.

5. *Unprepared Vegetable Tissues.*—There are some few substances which may, with more or less success, be cut into sections whilst in their natural condition. Such objects are to be found in the vegetable world, in certain kinds of leaves and allied structures, whilst in the animal kingdom they are principally represented by the various internal organs of man and the lower animals. Special directions are given in text-books for the preparation of sections of leaves and similar substances. For instance, it is recommended to lay the leaf, etc., on a piece of fine cork, and with a sharp knife to shave off thin slices, cutting down upon the cork. Another plan is to place the leaf, etc., between two thin layers of cork, and cut through the mass. No method, however, is at once so simple and successful as the process of imbedding in paraffin. To do this, it is

necessary to make a paper mould, by twisting a strip of stout writing-paper round a thin ruler, and turning in the paper over the end of the ruler. This mould, the height of which may vary from an inch to an inch and a half, should now be about half filled with melted paraffin mixture (§ 10), the leaf or other object plunged into it, and held in position by small forceps till the paraffin has become sufficiently solidified to yield it a support. More of the paraffin mixture is now poured in until the specimen is thoroughly imbedded; the whole is to be put away in a cold place for an hour or so, when the mass will be found sufficiently firm to be cut with ease. Sections may be made with a razor kept constantly wetted with water, or if the preservation of colour be no object, methylated spirit may be employed for the purpose. As the subsequent treatment of such sections in no wise differs from that required by those cut in the microtome, we shall defer its consideration until that method of section has been described.

6. *Unprepared Animal Tissues.*—For the cutting of fresh *animal* tissues several plans may be followed. Thus, if a section of only very limited area be required, it may be obtained by snipping a piece off the tissue with a pair of bent scissors, which for this purpose

are so made that the blades are curved on the flat. If this be carefully performed it will be found that a large portion of the section (particularly at its circumference) so obtained will be sufficiently thin for examination. If a larger section be required, an attempt may be made to cut it with a very sharp scalpel or razor, the blade of which whilst in use must be kept *flooded* with water or spirit, the latter of which is to be preferred. Recourse may also be had to *Valentin's* knife (Fig. 1). This

FIG. 1.—VALENTIN'S KNIFE.

consists of two long narrow blades, running parallel to each other, the distance at which the blades are held apart, and which, of course, determines the thickness of the section, being regulated by means of a fine screw passing through both blades. A milled head attached to this screw gives a ready means of opening or closing the blades, so as to bring them to the required degree of approximation. The method of using the knife is very simple. After having "set" the blades at the desired distance apart by means of the milled head, the tissue to be cut is held in the left hand, immersed in a basin of water. The knife is

now steadily and with rapid motion *drawn* through the tissue, care being taken that the cut is made in such a manner that the blades move from heel to point. By slightly separating the blades, and gently shaking them in water, the section at once becomes disengaged. After use, the blades must be thoroughly dried, when they may be smeared with some oil which does not readily oxidize. For this purpose a very suitable oil is that known as "Rangoon." Though it has been deemed advisable briefly to describe the preceding methods of cutting unhardened tissues, it will be found that, for the purposes of the ordinary microscopical student, sections so obtained are of little value. They are always of very limited dimensions, seldom of uniform thickness, and often so extremely friable as to render it very difficult, and frequently impossible, to submit them with safety to such further treatment as is necessary to fit them for being mounted as permanent objects. This method of section-cutting, however, is not without its uses, for by its means the medical practitioner is provided with a simple and ready method of roughly investigating the structure of morbid tissues, whilst to the general student it furnishes an easy means of making a cursory examination of certain sub-

stances, in order that he may determine whether it be worth his while to subject them to some of those various processes of hardening hereafter to be described.

7. *Cutting Hardened Tissues by Hand.*—Suppose we have on hand a specimen of animal tissue, hardened by the chromic acid method, which has just been described; let us proceed to consider the best means by which it may be cut into sections without the use of the microtome. The readiest and most simple plan, if the piece be large enough, is to hold it in the left hand, and having brought the surface to a perfect level by cutting off several rather thick slices, endeavour to cut a thin section by the aid of a very sharp razor, the blade of which must be kept well *flooded* with spirit. As in the use of *Valentin's* knife, so here, great care must be taken to *draw* the blade across the tissue, every effort being made to avoid *pushing* the knife, else the section will be *torn off* instead of being *cut*. Though this method is of very great importance for many purposes, yet a considerable degree of manipulative skill is required to enable the operator to obtain anything like perfect sections by its means, and unfortunately this skill is acquired by very few persons indeed, even after much practice. If the piece which it is desired to cut

be too small to be conveniently held in the hand, it may be imbedded in paraffin in the manner already described (§ 5). A very simple imbedding agent, and one of the greatest practical value, is a strong solution of gum arabic, which, upon being dehydrated either by ordinary drying or the action of alcohol, soon acquires such a degree of hardness as to permit it (with the imbedded tissue) to be easily cut. As this method of imbedding, however, is most frequently resorted to where, by its means, special difficulties have to be overcome, a full description of the process will be deferred until such special cases come to be spoken of.

8. *Microtome.*—Although the preceding plans may be sufficient to answer all his requirements, if the student wishes to obtain only one or two sections of small dimensions of a given object, yet, if he requires a number of such sections, he will find these methods fail him, for even though by practice he may have attained to considerable aptitude in the use of the knife, it will still unquestionably happen that the vast majority of his sections will be more or less imperfect. If, therefore, it be desired to procure a number of perfect sections of equable thickness and large area, it is absolutely necessary to resort to the use of some form or other of microtome, or section-cutter.

This instrument, in its simplest form, merely consists of a stout brass tube closed at one end, and being by the other fixed at right angles into a smooth plate of metal. A plug, or disk, of brass accurately fitting the interior of the tube, is acted upon by a fine-threaded screw, piercing the base of the tube, and by means of which the plug and any object it may support can be elevated at pleasure. The object by this means being made gradually to rise out of the tube, sections are cut from it by simply gliding a sharp knife along the smooth cutting plate, and hence across the specimen. Any intelligent worker in brass would make an instrument of this kind at a very small cost, and although perhaps it might lack the finish of an instrument bought at an optician's, it would, if accurately made, do its work as well as the most complicated and expensive. If, however, the student resolves to *purchase* a microtome, there are a variety of forms in the market from which he may choose. A few hints may perhaps be of service in enabling him to make a judicious selection. At the outset we may say, that unless the student intends to devote himself solely to the production of sections of wood, etc., he ought not to procure one of those forms of microtome known as wood section-cutters, in which the

object to be cut is held in position in the tube by means of a binding screw, which pierces its side. Although these machines act fairly well for cutting hard bodies, they are not at all suitable for soft ones; and even in the case of *wood*, if it be desired to preserve the bark perfectly intact, this form of microtome will not be found satisfactory, for, to give perfect steadiness to the object to be cut, it has to be so tightly jammed against the side of the tube that in emerging from it the sharp angle of the upper opening scrapes away a portion of the bark.

The chief points to be attended to in selecting a microtome are (1) that the cutting plate of the instrument be made of glass, or, in default of this, of very hard metal of the most perfect smoothness; (2) that the diameter of the tube be neither too large nor too small—it ought not to be less than five-eighths of an inch, nor greater than one inch; (3) that the screw, which should be *fine* and well cut, be provided with a graduated head; (4) that there be some kind of index by which fractional portions of a revolution of the screw may be measured; (5) that the plug fit the tube of the microtome so accurately that when melted paraffin, gum, or other imbedding agent is poured into it, it may not find its way between the plug and side of the tube; and lastly, it is of the utmost

importance that the microtome be provided with some kind of clamping arrangement, by means of which it may be firmly attached to the bench or work-table. Any instrument which is designed to stand *upon* the table, where it is supported by legs or other analogous arrangement, should unhesitatingly be rejected, for it is impossible to give to a construction of this kind that perfect steadiness which is so absolutely essential to satisfactory performance. Whatever other defects may be passed over in the selection of a microtome, this provision for ensuring perfect stability should be insisted upon.* It often happens in cutting tissues imbedded in paraffin that the pressure of the knife causes the cylinder of the imbedding agent to twist round in the tube of the machine, and so cause considerable difficulty and annoyance. This evil is usually met by running a deep groove across the upper surface of the plug, and into this the paraffin sinks, and so is prevented from rotating. It will be found, moreover, that another difficulty, of a kindred though much more serious character, will frequently be encountered. During section the

* These remarks do not apply to Williams' microtome, described in § 15. Its great size and weight render it perfectly steady when merely standing upon a table.

paraffin has a tendency, not only to rotate, but also to become loosened from the subjacent plug, and to *rise* in the tube of the microtome. When this happens the power to cut sections of uniform thickness has completely gone, for some will now be found to be many times thicker than others; in fact, the irregularity in this respect soon becomes so monstrous as to render it useless to prolong the sitting. In the

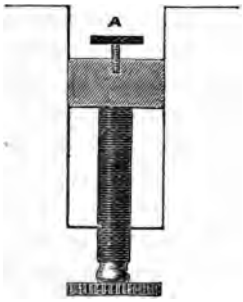

FIG. 2.—SECTION OF MICROTOME TUBE, SHOWING ARRANGEMENT A TO PREVENT RISE OF PARAFFIN.

ordinary run of microtomes no provision seems to have been made to meet this difficulty, and for this reason many instruments of otherwise great merit have their efficiency seriously impaired. Fortunately this imperfection is easily remedied, all that is required being that the upper surface of the plug should be fur-

nished with some kind of projection, having at its summit a table-like expansion, as shown at A in Fig. 2. The imbedding paraffin, by penetrating beneath and around this, becomes firmly attached to the plug, and thus all risk of its rising is effectually avoided. If the student wishes to secure a really first-class instrument, none can be so confidently recommended as the freezing microtome of Professor Rutherford, figured and described at page 43. In addition to its being the best instrument for carrying out the freezing method, this machine is equally effective for cutting tissues imbedded in paraffin, or any of the other agents used for that purpose; in fact, whatever work a microtome *can do, this one* will perform.

9. *Section-Knife.*—Of not less importance than the microtome is the section-knife to be used in conjunction with it. However perfect the former may be, and whatever the dexterity of the operator, unless he be provided with a suitable and well-made knife, he will never succeed in obtaining satisfactory results. As to the most desirable *size* of the knife much difference of opinion seems to exist, section-knives varying in this respect from a blade of extreme shortness to one which fell under our observation, in which the portentous length of *thirteen* inches was attained. What advantages

were to be expected by prolonging the blade to this extravagant length must remain an inscrutable mystery to all save its designer. Such a knife we were at one time deluded into working with, and can testify that the difficulties to be overcome in learning how to use so formidable a weapon without injury to oneself, more than counterbalance any possible superiority which a blade of this description could possibly possess over one of more modest dimensions. Concerning the *shape* of the knife, it is frequently advised that the surface which has to glide along the cutting-plate of the microtome should be ground *flat*—a most unsuitable arrangement, as a very little actual

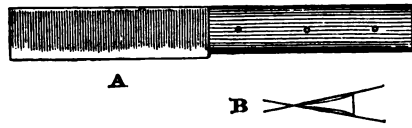

FIG. 3.—A, SECTION-KNIFE; B, TRANSVERSE SECTION OF BLADE.

experience of section-cutting will speedily demonstrate. After many unsuccessful attempts to obtain a really good and reliable section-knife, we determined to have one specially made, which, as it has proved everything that could be desired, merits a brief description.

It is of the utmost importance that the blade be made of good and well-tempered steel, not only that it may be capable of receiving the most exquisite keenness, but also that it may *retain it*. The knife of which we speak is here figured. It is furnished with a blade *four inches* long and seven-eighths of an inch broad, set in a square handle of boxwood, also four inches in length. The thickness of the blade at the back is not quite a quarter of an inch, whilst *both* of the surfaces are slightly hollow ground. It is essentially necessary that the back and edge of the blade be strictly parallel to each other; that is to say, that the edge must form a straight line, and both the edge and under side of the back must lie in the same plane, otherwise the knife, when in use, will have such a tendency to tilt over as to render its management extremely difficult. It is very easy to discover if this condition be fulfilled, for if, on carefully laying the flat of the blade upon a piece of level glass, every portion of both back and edge are found to be in close contact with it, the knife may, in this respect, be considered perfect. Every student who aspires to be a successful cutter of microscopical sections should provide himself with a good Turkey oilstone, *and learn how to use it*. This he may readily do by paying a few visits to the barber when he is engaged in

"setting" razors. By attentively observing how *he* goes about the work, the student will speedily acquire a bit of practical knowledge, the value of which he will not be long in discovering. He should also possess a razor-strop, as it will be in constant requisition. It may here be remarked, that though *razors*, as a rule, are unsuitable for use with the microtome from want of uniformity in the thickness of their blades, yet if only a small object is to be cut—for instance, a thin root, or stem—very good results may be obtained by their use, especially if one of the old-fashioned make, having a thick back and slightly *concave* surface, be employed.

10. *Imbedding in Paraffin for Microtome.*—Having described at some length the various instruments necessary for section-cutting, we will now consider how they are to be used. Let us endeavour to cut some sections—say, of a piece of kidney—and in so doing we will adopt the "paraffin" method of imbedding. Ordinary paraffin, however, when used alone, is rather too hard for our purpose. In order, therefore, to bring it to a suitable consistence it must be mixed with one-fifth its weight of common unsalted lard, a gentle heat applied, and the two substances thoroughly stirred together. In the cold weather of winter, even

this mixture will often be found too resistant; in that case the above proportion of lard must be slightly increased. A quantity of the mixture should be prepared so that it may always be ready when wanted—it is very conveniently kept in an ointment pot, or preserve jar, the top of the latter being well covered to keep out the dust. When it is intended to use the mixture for the purpose of imbedding, only just about the quantity likely to be required should be melted; for in doing this it is advisable to use as low a degree of heat as possible, not only to prevent injury to the tissue to be imbedded, but also that the paraffin on cooling may not undergo such an amount of contraction as to cause it to shrink from the sides of the microtome tube. It is, therefore, a good plan to effect the melting in a water-bath, a simple kind of which, something after the fashion of a glue-pot, would be made for a few pence by any tinman.*

The kidney which we are about to cut has, of course, gone through the process of hardening already described, and is now preserved in spirit. A small piece, say, half an inch square,

* Small glue-pots made of tin, filled with glue and provided with a brush, may be purchased at most ironmongers and chemists for about sixpence each. When the glue has been removed, such a pot makes a very handy water-bath in which to melt paraffin.

is selected, removed with forceps, and placed upon a bit of blotting paper, when the surface of the tissue will rapidly become dry; *only the surface* must be allowed to dry. It is the usual plan, now to proceed at once to imbed it in the melted paraffin. This is a most undesirable step, and gives rise, at a later stage of our proceedings, to a great amount of trouble and annoyance, for after sections have been cut from a tissue so imbedded, it will be found that portions of paraffin adhere to their edges with such tenacity, that in the case of many of them, there is no effectual method of removing the paraffin short of soaking the sections in warm ether, an objectionable and expensive proceeding. All this annoyance may be prevented by subjecting the tissue to a simple preparatory treatment before it is imbedded in the paraffin. For this purpose prepare a *very weak* solution of gum arabic in water—twenty grains to the ounce. Into this, by means of the forceps, dip for a few moments the already *surface-dried* tissue, taking special care not to squeeze it, or the pressure will cause the spirit from its interior to remoisten the surface, which would prevent the gum from adhering. We shall see the value of this a little later on. Remove the tissue from the solution on to blotting paper, when the superfluous gum will speedily

drain off, and in two or three minutes the surface will become quite glazed and dry. Having melted some paraffin mixture in the water-bath, the tissue, held in the forceps, must be plunged for an instant into the heated liquid and immediately withdrawn, when the crust of paraffin with which it is enveloped will promptly harden. Whilst this is taking place, we may make ready the microtome. Having by means of the milled head or handle depressed the plug in the tube so as to leave a free opening about an inch deep at its upper end, we must pour in the melted paraffin, which by this time will have become a little cooler, until the cavity be about half filled. The prepared tissue must now be introduced, care being taken to place it in such a position that the sections may be cut in the desired direction. The tissue must, if necessary, be held in position with forceps or a needle point, till the imbedding material becomes hard enough to give it due support. It is here to be remembered that it will not be advisable to place the tissue in the centre of the tube; it will be much more easily cut if placed nearer to that edge of the tube which is situated next to the operator in the act of cutting (see Fig. 4). More paraffin is to be slowly added until the tissue is completely covered; even after

this, still more should be added, for it will be found that in cooling the paraffin shrinks, so as to leave a cup-shaped depression in its centre, whereby portions of the tissue which were previously covered are again laid bare. The best method of preventing this is to use the paraffin at as low a temperature as possible, and to use plenty of it. The microtome, with its contents, must now be removed to a cool place, when the paraffin will soon become solidified. Whilst this is being accomplished we may make our further preparations. The first thing we require will be a large basin, full of freshly filtered water, and provided with a cover. A small beaker of methylated spirit, with a dipping rod or pipette, will also be necessary. We must now see that the section-knife is in thorough order, to ensure which it will be advisable to give it a few turns on the strop. An ordinary razor will also be of service.

11. *Employment of Microtome.*—The paraffin being sufficiently hard, we will clamp the microtome on to the table, and seat ourselves upon a chair of convenient height before it. To our right stand the basin of water, razor, and section-knife, the beaker of spirit to the left, and a cloth on our knee. A few turns of the microtome screw having brought the paraffin to the surface, a thick slice is to be cut off, and this proceeding

repeated until the imbedded tissue comes into view. This preliminary work had best be done with the razor, as it is needless to subject our section-knife to unnecessary wear and tear. By a fractional revolution of the screw the tissue is now slightly elevated, and from the pipette, held in the left hand, a large drop of spirit is to be

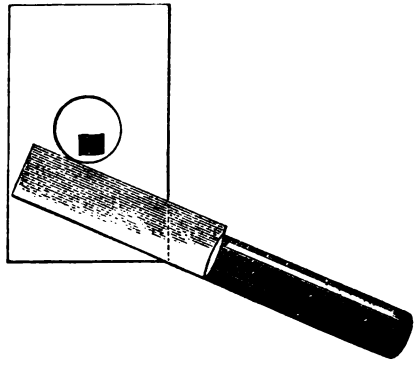

FIG. 4.—DIAGRAM SHOWING POSITION OF KNIFE IN COMMENCING TO MAKE A SECTION.

let fall upon its surface. The section-knife, grasped firmly but lightly in the right hand, is to be laid flat upon the cutting plate of the machine, so as to occupy the diagonal position shown in the figure. Two fingers of the left hand are now laid gently upon the back of the blade, so as to give it an equable support, whilst

the knife with a rapid motion is pushed in the combined direction of *forwards* and *to the left*, so that the blade in cutting the tissue will pass through it from point to heel. Thus it will be observed that the stroke of the knife is *from* the operator—a far easier mode of cutting than the reverse plan. The blade of the knife, having the section just cut, either floating in a pool of spirit upon its surface, or adhering thereto, must now be dipped into the basin of water, when by a little very gentle agitation of the knife the section will be floated off. And now we shall find the great practical value of immersing the tissue in gum before imbedding, for no sooner is the section disengaged from the knife than the thin film of gum which separates the paraffin from it becomes dissolved, and the section will be observed gradually to subside to the bottom, leaving the paraffin floating upon the surface of the water. After carefully wiping the knife from all shreds of paraffin, the microtome screw must again be partially rotated, more spirit applied to the tissue, and another section being cut, it must be transferred to the water as before, and so on, until a sufficient number of sections have been obtained. As to *how thin* the sections must be cut, no general directions can be given; each case must be regulated by its own conditions—the denser

the tissue the thinner should the section be; whilst certain substances of loose and spongy texture do not require the sections to be particularly thin—it may be said, however, in a general way, that sections, and especially animal ones, *cannot be cut too thin*, so long as they remain perfect and entire.

12. *Freezing Microtomes.* — Our preceding consideration of the method of employing the microtome in conjunction with paraffin as an imbedding agent, will have formed a very suitable introduction to the study of the somewhat more complicated process of imbedding the tissue in gum, for section in the freezing microtome. This method is of the utmost value to the practical histologist, for by its means he is enabled with ease to possess himself of perfect sections of several structures, the cutting of which, before the introduction of this process, was always a matter of difficulty and anxiety. The freezing microtome is especially valuable for the section of such substances as, from their extreme delicacy, are liable to be injured by being imbedded in paraffin—for instance, the delicate villi of the intestines become very frequently, by the use of paraffin, denuded of their epithelium, and the villi themselves not seldom become torn off or otherwise damaged. The great value of the method is

also very well seen in the treatment of those tissues which, like the lung, are of such loose and spongy texture as to offer insufficient resistance to the knife unless their interstices have been previously filled up with some solid yet easily cut material. As the space at our command is strictly limited, we are precluded from entering into this branch of section-cutting as fully as the importance of the subject demands, and our own inclination would lead us. To those who wish to become conversant with the full value of this method, we cannot do better than recommend the perusal of Professor Rutherford's *Practical Histology*, second edition, than which, on the whole subject of physiological microscopy, no treatise in the English language with which we are acquainted, is at once so plain, practical, and profound.

13. *Rutherford's Freezing Microtome.*—This, like the microtome already described, has for a basis a horizontal cutting plate, into which is inserted at right angles a long brass tube, in the interior of which works a fine-threaded screw, by whose action the "plug," bearing upon its surface the object to be cut, can be raised and lowered at pleasure. A large box or chamber (see A, Fig. 5) is so arranged as to surround the upright tube, so

that when this chamber is filled, in the manner described further on, with a freezing mixture, heat is rapidly abstracted from the tube and any object it may contain. Thus, soft and delicate objects imbedded in mucilage and placed in the "well" of the microtome, soon become, together with the imbedding agent, sufficiently frozen and solidified to render their

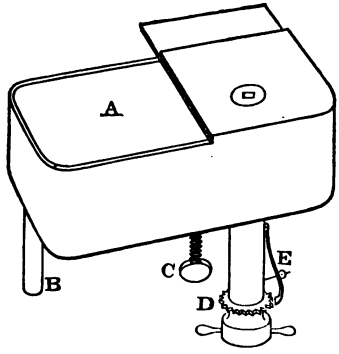

FIG. 5.—RUTHERFORD'S FREEZING MICROTOME.

section easy. The cutting plate is prolonged at the back of the microtome for some little distance beyond the upper surface of the freezing chamber; and here, in combination with another brass plate jutting out from the under surface of the microtome, it forms a clamping arrangement, by means of which, with the aid of the screw C, the microtome may be securely

fastened to a bench or table. In the most recent makes of the instrument the upper surface of the cutting plate is covered with plate glass—a very valuable improvement upon the older styles. In order to prevent interference with the freezing process, by the admission of external heat, the bottom and sides of the freezing chamber are covered with sheet gutta-percha, which is firmly cemented to them. An outlet, or waste-pipe, for the exit of the freezing mixture, as it liquefies, is provided at B. To register fractional portions of a rotation of the micrometer screw, the head of the latter is graduated by means of a series of pin holes, into which, as the screw rotates, a sharp point connected with the spring E drops. When the microtome is used for freezing it will, as a matter of fact, be found that this arrangement is imperfect, for during the process the whole of the microtome becomes so covered with hoar-frost, resulting from the condensation and freezing of moisture from the atmosphere, that the sound produced by the *click* of the spring is so deadened as to become nearly inaudible. If, as is shown in the figure, at D, a cogwheel be substituted for the row of perforations, and a triangular wedge be attached to the spring in place of the needle point, it will be found, when the screw is rotated, that

as each tooth of the wheel passes under the spring, a loud and distinct click will be given out. This little modification may be considered to be scarcely worth mentioning, but its *practical value* will not be questioned by those who give it a trial.

14. *Use of Rutherford's Microtome.*—A very suitable object with which to demonstrate the method of using this form of microtome will be afforded us by a piece of the intestine, say, of the ileum of a cat or dog. Suppose we have some of this, which has been previously hardened in the manner already described, now lying in methylated spirit. Let us select a piece about half an inch in length. Our first care will be to deprive it of its spirit; for so long as the tissue remains impregnated with alcohol it would, of course, be impossible to freeze it. We will, therefore, throw it into a large basinful of water, and leave it there for twenty-four hours, during which time it will be as well to change the water once or twice. We shall now require a strong solution of gum. This, which should have been made some time previously, may be prepared by placing a quantity, say, three or four ounces, of ordinary gum arabic in a glass beaker, and adding sufficient water to cover it; the mixture must be stirred occasionally with a glass rod until

solution has taken place, which will be in a few days. Sufficient water may now be added to bring the solution to the consistence of *thin* syrup.* The gum must now be sieved through a piece of fine cambric, which has previously been wetted with water, into a wide-necked bottle provided with a *loosely* fitting cap, to prevent the entrance of dust. Mucilage, by keeping, is very apt to become sour and mouldy; this may be prevented by adding to each ounce of the water with which it is prepared about half a grain of salicylic acid. We now pour some of this mucilage into a small vessel—an egg cup will answer very well—and into it transfer the piece of ileum from the water. Here we must allow it to remain for a time sufficient to permit of its becoming thoroughly saturated with the gum, for which purpose some hours will be necessary. Owing to the high specific gravity of the mucilage, some little difficulty may be experienced in getting the tissue to sink down into it. To overcome this, the specimen should occasionally be pushed overhead, until it absorbs sufficient of the gum to keep it submerged. When the soaking has

* In the first edition of this book very thick mucilage was recommended. Subsequent experience, however, shows that better results are obtained by using such a solution as the one now described.

been accomplished we will prepare the microtome. In the first place, it will be necessary to remove the plug, which is to be done by turning the handle connected with the screw until the plug rises so high in its tube that it may be grasped with the fingers and removed, when it is to be well smeared all over with sperm oil and replaced. This is done to prevent any unpleasant adhesions taking place whilst the freezing is going on. We must now depress the plug so as to convert the upper part of the tube into a kind of "well" of sufficient depth to hold our specimen. It will now be very advisable to look carefully into this *well* and observe whether the plug fits accurately into the tube, for if there be any appreciable interval between the two it will give rise to much subsequent annoyance, as the gum penetrating this interstice will have become firmly frozen into irregular patches, which will so interfere with the even gliding of the plug within its tube as to cause the former to ascend in such an irregular and jerky manner as to be utterly destructive of all accuracy in the cutting. If this defect be observed it may be at once remedied by dropping a small quantity of gently heated paraffin into the *well*, which will effectually close up any fissures. The microtome, by means of its clamping arrangement, must

now be firmly attached to the table, and a suitable vessel be placed on the floor beneath it, so that it may catch the water which will issue from the waste-pipe of the apparatus. The next requirement is a supply of block ice and finely powdered salt. A lump of the ice must be wrapped in a towel and crushed into small pieces; these, by means of a large mortar, are to be further reduced to a very fine powder. Any attempt to hurry over this troublesome part of the operation will lead to future disappointment, for unless the ice be used in a very fine powder, great delay (at least) in the freezing will be the result. With the aid of a small spoon the ice and salt are, in alternate spoonfuls, to be conveyed into the freezing box of the machine, great care being taken that the cavity under the cutting plate and around the tube be thoroughly packed, after which the uncovered portion of the box should also be well filled. The *well* is now to be filled with the mucilage to within a little distance of its top, and a piece of sheet gutta-percha (such as shoe soles are made of) being applied over the well and kept in position by a weight, we must wait until the freezing commences. In a short time we shall, on removing the gutta-percha for that purpose, see that the gum has acquired a thick muddy appearance. The tissue must

now, by means of the forceps, be transferred to the well, and there placed in such a position that the sections, when cut, shall run in the desired direction. After more gum has, if necessary, been added, so as completely to cover the tissue, the *well* is again to be covered and attention given to the freezing box. As the mixture which this contains becomes melted it must constantly be renewed, care being at the same time taken that the mouth of the discharge pipe be kept quite free, otherwise, water accumulating in the box, the freezing mixture will degenerate into a useless puddle. When the gum has become sufficiently hard for cutting, this must be done much in the same manner as if paraffin has been used (§ 11). In this case, however, no fluid will be required, or must be used, to wet the knife with, and especial care must be taken that in disengaging the sections from the knife into the water they be not torn. These sections often adhere very tenaciously to the blade, but if a little patience be exercised the water will soon float them off in safety—much more safely than if any attempt be made to liberate them prematurely. It occasionally happens, and especially when the weather is very cold, that the frozen tissue becomes so extremely hard as to divert the course of the knife, and by tilting the edge of the blade

upwards causes it to pass *over* instead of *through* the specimen. To overcome this difficulty the knife may be slightly warmed by occasionally plunging the blade into a basinful of hot water, or (a course which answers better) the cutting may be suspended for a little while, until, by the partial thawing of the material, a more suitable consistence has been obtained. This inconvenience may also be materially lessened by previously soaking the tissue to be cut in a mixture of one part by measure of glycerine to thirty of mucilage, and then imbedding and freezing in *pure* mucilage in the manner already described. There is another circumstance connected with the use of the freezing microtome which is rather annoying. The moisture of the breath and atmosphere is apt to become condensed on the cutting plate, and here, mixed with accidental smears of gum, it becomes frozen into a jagged and irregular sheet of ice, which not only seriously interferes with the smooth play of the knife, but also constitutes a real peril to its edge. This nuisance may be avoided by keeping the cutting plate smeared with pure glycerine, taking special care that none of the glycerine comes in contact with the frozen mucilage in the *well*. When using the freezing microtome it is always advisable to wear an apron, otherwise our clothes may

receive considerable damage from the constant splashing of the salt water as it falls from the waste-pipe into the vessel beneath it. After use, the microtome must be well washed in plenty of cold water until every trace of salt be removed, for if any of this remains it will quickly corrode the brasswork of the instrument. The plug and screw, as also the section-knife, should be well smeared with Rangoon oil before the apparatus is put away.

15. *Williams' Freezing Microtome.*—As will be seen from Fig. 6, this microtome, though, like Rutherford's, designed for use with salt and ice, is constructed on an altogether different plan. The machine consists of a large round wooden box, the interior of which is coated with pitch or asphalte, in order to render it water-tight. From the centre of the bottom of the box arises a massive pyramidal upright made of brass, and terminating at its apex in a screw thread, upon which small brass disks, supplied with the microtome, fit. The box is closed with a tightly-fitting lid, which, upon its upper surface, is covered with smooth glass. The centre of this lid is perforated for the passage through it of the brass pyramid just spoken of. To prepare the microtome for use, the wooden box must be packed with a mixture of salt and ice, precisely in the same manner as

described when speaking of Rutherford's microtome. When the box is nearly full, the mass should be firmly pressed down into it and around the upright, and the lid then tightly fitted on. To use the microtome we take a piece of tissue already prepared in gum, in the manner previously described, and having screwed on one of the disks to the end of the upright, we put our tissue on this disk and let fall around it a few drops of mucilage, in such a manner that, when frozen, it will form a supporting wall around the tissue. When the specimen has become frozen, which will be in a very short time, how are we to cut it? As no means are provided for propelling the tissue upwards towards the knife, it has been necessary so to arrange the knife that it may be made to descend upon the tissue. This is effected by fixing the blade of the knife into a triangular framework of brass, through each angle of which a screw with a very fine thread passes, so that when the frame carrying the knife is laid upon the glass cutting-plate of the microtome, both knife and frame are elevated at some little distance above it, this distance being regulated by the length of the screws. Suppose the tissue on the brass disk to be now frozen, let us proceed to use the knife. First, by means of the screws, we regulate the height of

FIG. 6.—WILLIAMS' FREEZING MICROTOME.

[*See page* 51.

the framework, so that on passing it over the disk the edge of the knife just *clears* the tissue to be cut. A slight turn is now to be given to the graduated head of the large screw in the apex of the triangle, which will slightly depress the knife edge. The frame, supported by its screws, which, being tipped with ivory, glide over the smooth glass readily, is now to be pushed forwards over the tissue in such a manner that the edge of the knife may pass diagonally across it, and thus remove from its surface a section. The latter having been removed by means of a camel-hair pencil and transferred to water, the upper surface of the knife may be smeared with mucilage, and as many more sections cut as may be required. The advantages possessed by this microtome are three: (1) The tissue, when once frozen, remains so for an amazingly long time; (2) the edge of the knife never coming in contact with anything but the tissue to be cut, does not readily become blunted; and (3) less practice is required to use it than the ordinary microtome demands.

The only objection there appears to be to the microtome is, that each section, as cut, has to be removed from the knife by means of a camel-hair brush, a process hazardous to the integrity of the section. When we have used

the microtome we have obviated this by removing, after each section, the blade from its frame, and allowing the section to float off the knife into a basinful of water.

With this microtome is also supplied a shallow cylinder capable of being attached to the central upright in place of the brass disks. In this cylinder objects may be imbedded in paraffin or other substances, and the freezing microtome be used as an ordinary one.

16. *Ether Microtomes.*—Whilst using the ordinary forms of ether microtome the operator must necessarily be exposed to an atmosphere highly charged with ether vapour, which in most people tends to produce headache and other inconveniences, and even to some it is very possible that its continued inhalation may prove positively dangerous. For this reason, and because they have been superseded by more perfect instruments, all description of them has here been omitted. Dr. J. W. Groves has adapted Williams' freezing microtome (already described at page 51) for use with ether instead of salt and ice, and the result of this adaptation is, that when the microtome is used with ether none of the inconveniences just mentioned are experienced.* For this

* *Journal of Quekett Microscopical Club.* October, 1881, *p. 293.*

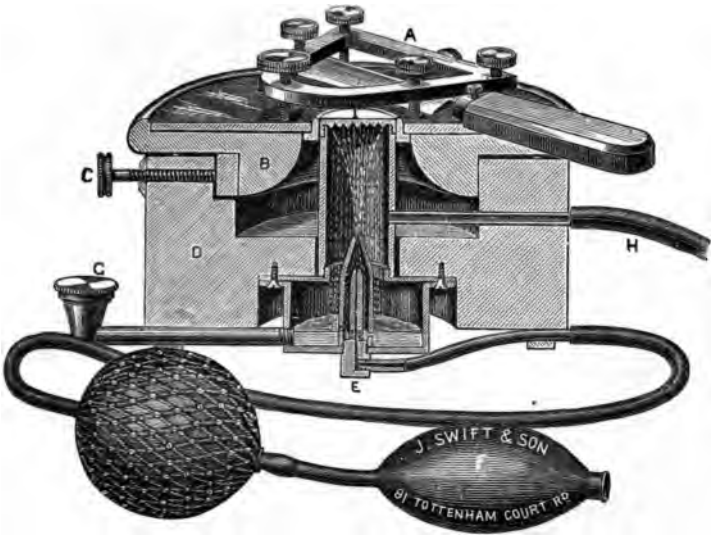

FIG. 7.—VERTICAL SECTION OF THE GROVES-WILLIAMS' ETHER FREEZING MICROTOME.

[*See page 56.*

purpose (see Fig. 7) he has substituted for the pyramidal upright in the centre of the freezing box a hollow cylinder, having for its lid, I, a flat plate on which the object to be frozen lies. At the base of this cylinder is a chamber which can be filled with ether by means of a tube opening into it, which tube, at its other extremity, is closed with a metal cap, G. Through a hole in the bottom of the chamber there also passes vertically upwards a small tube terminating above in a finely perforated cone, and having its base plugged up. Through this plug, E, there passes also vertically upwards another fine tube, to within a little distance of the conical head of the outer tube. If the ether chamber be charged, and the continuous action bellows, F, of an ordinary spray producer be now connected with the base of this inner tube and the bellows set to work, the current of air rushing violently along this tube and through the cone of the outer one, a partial vacuum is produced between the two tubes which is immediately filled by an inrush of ether through a hole in the outer tube provided for that purpose. This ether being caught in the rush of passing air, is carried upwards through the cone and becomes projected in the form of spray upon the under surface of the lid of the cylinder, which, as

before said, forms the plate upon which the object lies. The greater portion of the ether here becoming condensed, falls back into the ether chamber to be used again and again. A small percentage of ether is, however, lost, for which an escape is provided by means of a tube, H, passing from the chamber to the outside of the machine, where by india-rubber tubing it may be prolonged so as to reach out of doors; thus the deleterious effects produced by the diffusion of escaping ether into the atmosphere surrounding the operator, are entirely avoided.

In the microtome just described, it will be remembered that the sections were cut and their thickness regulated by the gradual descent of the knife towards the tissue to be operated upon. In order to reverse this process, and provide a machine in which the tissue shall ascend towards the knife—as is the case in the ordinary form of section-cutters—Messrs. Swift and Son have brought out their *new improved microtome*, a drawing of which is given in Fig. 8. The instrument consists of a massive iron upright, terminating at its lower extremity in a clamping arrangement, by which the microtome may be securely fastened to the work-table. From the top of the upright two highly-polished iron bars, lying parallel to each other, run horizontally forwards. These bars correspond to

FIG. 8.—SWIFT AND SON'S IMPROVED MICROTOME.

[See page 60.

the *cutting plate* in the usual form of microtome, and upon them, as will be seen at A in the drawing, a flat brass frame carrying a knife is made to glide. The knife is kept firmly in position on this framework by means of the binding screw C, the end of which, terminating in a square clamp, presses against the back of the blade. The face of this clamp is grooved in different directions in such a manner that, according as the back of the blade is received into one or other of these grooves it is pushed from or drawn towards the level of the framework, thus affording a means by which the edge of the knife may be set at varying angles to the tissue to be cut. In front of the iron stand will be seen an angular upright pillar carrying in front of it a short length of sprung brass tube, B, into which any of the apparatus, presently to be described, may be firmly fixed by a clamping screw. By means of a micrometer screw, E, fixed at the base of the angular pillar, the sprung tube, and of course whatever it may carry, can be acted upon so as to raise or lower it at pleasure. The amount of movement thus effected is registered by the milled head of the screw, for which purpose three concentric circles have been drawn upon its face, each of which is so graduated that, as the face rotates from mark to mark, the distance tra-

versed by the screw, and which of course determines the thickness of the section, will in the case of the outer circle be 1000th, in that of the middle 500th, and in the inner one 400th of an inch. The index by which these measurements are recorded consists of a spring catch so fitted that, as the milled head rotates, it drops into the divisions of the circles, into either of which it can be shifted at pleasure, or, if desired, can be thrown out of gear altogether.

When it is intended to use the microtome for freezing with ether, the chamber provided for that purpose, and which in the engraving is shown in position, must be employed. This chamber is exactly like the one already described when speaking of the Groves-Williams' microtome, and consists of a reservoir for containing the ether, and an upright cylinder leading from it, and terminating in a flat plate upon which the object to be frozen lies. To use the machine, remove the cup, D, fill the chamber with ether, then fix the cylinder in the clamp, B, when the bellows, F, being worked the ether will be projected through the tubes in the interior of the chamber (and which were described at p. 59), upon the plate holding the tissue, with the effect of speedily freezing it. When, under the action of the micrometer screw, the object to be cut has moved upwards between

the cutting bars sufficiently high for the purpose, sections are to be obtained by simply pushing the frame carrying the knife obliquely across the bars and through the tissue. In what manner such sections are afterwards to be dealt with has already been explained. For freezing purposes, common methylated ether of a density ·720 answers perfectly well. In winter, when ice is plentiful, and where only a very small piece of tissue requires to be frozen, the freezing may be effected without the employment of ether. For this purpose it will be necessary to use Dr. Pritchard's *solid freezer*, which is shown at the lower left hand corner of the engraving. As will be seen, it consists of a solid metal block, having its upper surface, upon which the tissue to be frozen lies, roughened so as to prevent the specimen from slipping during section. For use, the block and tissue are frozen by being immersed in powdered ice and salt, then the block is secured in the clamp, B, and sections cut in the manner just described.

The microtome, though essentially a freezing one, may, however, be employed for cutting objects imbedded in paraffin. For carrying out this, the box shown in the drawing at the lower right hand corner, has been provided. The tissue is to be imbedded in this box, in the manner described at page 36, and when the

paraffin has become quite cold, the box must be secured in the clamp B, and the tissue sectionized.

Yet another piece of apparatus belongs to this machine. It is called an *adjustable vice*, and will be seen at the foot of the engraving in Fig. 8. It is a most useful accessory, and there has long been a want felt for something of its kind. It consists of a cylinder carrying at its upper end the two jaws of a vice. One of the jaws is fixed, whilst the other, being moveable, may be made to recede from or approach to its fellow by means of the screw, so that hard substances of different kinds and various sizes may be securely fixed and held between the jaws, when, the cylinder being inserted in the clamp B, sections may readily be obtained. To the really working microscopist, this little appliance will be found of infinite value in a thousand directions. The uses of it are so obvious that no words will be wasted in describing them.

In the three microtomes last described, the section-knife, when in use, is mounted on a frame. In the Williams' and Groves-Williams' machines this arrangement has been rendered necessary by the very construction of the microtomes, but in Swift's improved form, though the same method is carried out, no absolute neces-

sity for its adoption exists, for the construction of the microtome permits of the use of a simple unmounted knife as readily as one mounted on a frame. No doubt the frame arrangement has some advantages over the simple knife, for, as before pointed out, the edge of the blade, coming into contact with nothing but the tissue to be cut, retains its keenness for a considerable period, whilst to the inexperienced operator, the extent of surface which the frame covers, and the readiness with which it glides over the cutting plate, gives a confidence which renders the use of the knife so guarded comparatively easy. On the other hand, the arrangement cannot but be looked upon as a somewhat clumsy one, and is open to the grave objection that it renders the disengagement of the sections from the knife both a tedious and unsafe process. Altogether, after having given both plans a fair trial, the author is strongly of opinion that, in the hands of one who by careful practice has taught himself how to use it, a simple unguarded knife of suitable shape and size is to be preferred to any mechanical arrangement whatever. To this conclusion he has been led by the teachings of a very considerable amount of practical work, and but that it would occupy too much space and perhaps be rather out of place here, he could demonstrate

that upon theoretical grounds also his conclusion is a sound one.

17. *Etherized Paraffin Method.*—As, by the freezing process certain substances of a soft and spongy nature, or which contain cavities, can, by having their interstices filled up with mucilage, which on being frozen becomes solidified, be brought to such a condition that their section is rendered easy, so, the same result may be attained by substituting for the mucilage, paraffin held in solution by a volatile solvent, on the evaporation of which, the solid paraffin being left in the interstices of the tissue, fulfils there the same purpose as the frozen and solidified gum. The substances to which this process is applicable comprise all objects derived from the vegetable kingdom, as also such animal tissues as contain little fat, *or where the removal of this substance* from the section would detract little or not at all from its value; and this is *practically* the case with a very large number of animal tissues. For the purpose of carrying out the method, the specimen is to be hardened in the usual manner, and after having lain in alcohol for a little while, it is to be transferred to a tightly-stoppered bottle containing *methylated* ether, where it may remain for an hour or two. Now, into a wide-necked bottle pour a little ether, immerse the bottle in

a basin of warm water, and add *thin shreds* of paraffin to the ether, until it will dissolve no more. Into this mixture transfer the tissue from the ether, *lightly* cork the bottle,* and let the specimen remain there for half an hour or so, the mixture being meanwhile kept fluid by the introduction of more warm water into the basin. The preparation may now be removed, allowed to become quite cold, then imbedded in paraffin and cut in the manner already described. The tissue will be found to be thoroughly permeated with solid paraffin, and very thin sections even of spongy and delicate preparations may readily be made. The sections, as cut, may be transferred either directly into methylated spirit, or, for convenience, may be first floated off the knife into water—in any case they must be allowed to soak for a short time in alcohol, after which they are to be transferred to a test tube of suitable size which has *already* been about half filled with spirit. After a little time the alcohol is to be drained off, sufficient ether added to a little more than cover the sections, and the tube immersed in water warm enough to make the ether boil.† This is

* To prevent all risk of explosion, it is well to interpose a piece of thick string between the cork and the neck of the bottle, so as to give exit to the evaporating ether.

† The boiling point of ether is so very low, that the temperature thus reached will not damage the sections.

then poured off, fresh ether added, and the process repeated for two or three times, in the last stage spirit being substituted for ether. The sections may be stained and mounted by any of the methods which will be described further on. Very little ether need be wasted, for that used for washing the sections may again be employed in the preliminary soaking, or for preparing the etherized paraffin.

It must be distinctly understood that this process is not proposed as a rival to the freezing method. Good sections can be obtained by its use, but when that method is practicable, the freezing process is to be preferred. Freezing microtomes are, however, of necessity somewhat expensive, and it is for this reason that the etherized paraffin method is here described, in order that a substitute, though a humble one, for the freezing process may be placed in the hands of those students—and there are many such—to whom a freezing microtome is an inaccessible luxury.

18. *Miscellaneous Imbedding Agents.* — In addition to the various methods of imbedding already described, many others have been proposed to meet special and exceptional cases. Thus, for imbedding young embryos and similar soft tissues, Duval* recommends that

**American Quarterly Microscopical Journal*, July, 1879, p. 323.

they should be hardened by the methods previously mentioned, stained with carmine, and then placed in alcohol. From this they are to be transferred to ether for a few moments, and then to pure *collodion*, in which they may remain from ten minutes to twenty-four hours; from this they are removed to alcohol. The collodion here solidifies without contraction, and all the parts of the tissues are retained in their normal position. Thus prepared, the specimen may be cut into sections at once, or preserved indefinitely in alcohol. The sections may be mounted in glycerine without removing the collodion, as it is so transparent that on microscopical examination its presence will not be noticed.

Insects enclosed in chitinous cases may, according to Hyatt,* be advantageously imbedded in *shellac*. For this purpose the insect is to be placed in alcohol and allowed to remain there until it is thoroughly permeated by the spirit. It must then be removed to a clear alcoholic solution of shellac, in which it may remain for a day or two. A cylinder of soft wood is now to be so prepared that it will fit accurately into the tube of the microtome. This cylinder is now to be split longitudinally down the middle, and a groove cut into one or both of

* *American Monthly Microscopical Journal*, January, 1880.

the half cylinders sufficiently large to admit the object without pressure. The two halves, with the object enclosed between them, are now to be fastened together with thick shellac varnish, and a thread passed around them to keep them in position. In a day or two the shellac will become quite hard, when the cylinder is to be soaked in warm water to soften the wood, then placed in the microtome, when thin sections may readily be obtained from it. If the sections should be rendered so opaque by the presence of the shellac as to interfere with their satisfactory examination, the addition of a few drops of a solution of borax will soon render them transparent.

Very soft tissues may also be imbedded in *white of egg*.* The material, freed from alcohol, is to be placed in white of egg in a little paper box, and the whole exposed to heat until it becomes hardened. Sections are then to be cut, stained, passed through alcohol and oil of cloves with balsam in the usual manner. In the balsam, the albumen contained in the section becomes clear and transparent.

Soft and spongy tissues may also be imbedded and their pores and cavities filled up by the use of an alcoholic solution of *soap*.† Twenty-

* *American Quarterly Microscopical Journal*, January, 1879.

† *Journal Royal Microscopical Society*, vol. ii. p. 940.

eight grammes of shavings of stearate of soda soap are to be dissolved in a water bath in 100 c.c. of 96 per cent. alcohol, and to the liquid mass water is to be added gradually, until a drop of the mixture let fall into a watch-glass quickly forms a transparent coagulum. To effect this, from about 5 to 10 c.c. of distilled water will be required. When the mixture, which should be kept in a stoppered bottle, is required for use, the mass should be melted by the aid of heat, the object to be cut immersed in it, and when cold the specimen may be cut in the usual manner. For the purpose of withdrawing the soap from the sections, they must be steeped for some time in pure methylated spirit.

The author is bound to confess that of the practical value of the methods just mentioned he has had very little experience; every want required by the class of work in which he has been chiefly engaged having been amply met by the various processes which have previously been described. As, however, the field of microscopical study is very wide, no doubt there are many students in its various departments to whom these methods, or the suggestions to be drawn from them, may prove of value, hence their insertion here.

19. *Preserving Sections.*—For many purposes

it is necessary that sections should be left in water for long periods. In very hot weather it will be requisite, to prevent the development of bacteria and the occurrence of putrefactive changes, to add to the water a few drops of pure carbolic acid. It will often happen that sections will accumulate to so great an extent that it is inconvenient or impossible to mount them at the time. In such a case they may be preserved unaltered for a very long time in a mixture of equal parts of methylated spirit and distilled water, the bottle in which they are kept having its neck and cork thickly smeared over in every spot with melted bees'-wax, in order to prevent evaporation of the spirit. Sections may also be kept microscopically unaltered for (it would appear) an indefinite period in a solution of forty grains of chloral hydrate to one ounce of distilled water.*

* "A Treatise on Therapeutics," by H. C. Wood, Junr. M.D., New York, 1877, p. 325.

PART III.

On Staining Animal Sections.

20. *Staining Agents.*—Having, by some one of the various processes just detailed, obtained our sections, it will be very advisable before proceeding to mount them that they should be submitted to the action of some staining agent in order to render more clear and distinct their minute structure. Organic substances possess the property of being able to absorb various colouring matters from their solutions, and to incorporate such colour into their own texture. This power of attraction is not, however, possessed by all substances indiscriminately, or to an equal extent. Some possess it in a high degree, whilst others appear to be nearly, if not entirely, devoid of such power. Hence it follows that if we immerse an organic tissue (one of our sections, for instance) of complex structure in a suitable staining fluid, the tissue will not become stained in an even and uniform

manner throughout, but the several portions of it will receive varying depths of colour in accordance with the varying attractive power of its several constituents. By this means we are enabled, in stained sections, to discriminate, by their difference of shade, minute and delicate structures, which, in the unstained condition, it would be difficult, and often impossible, to differentiate. For the purpose of section-staining there are many agents in use, the most generally suitable of which, for ordinary purposes, are carmine, logwood, and the double stain of carmine and indigo carmine, the preparation and use of which stains are now immediately to be described; whilst, for special purposes, various other dyes, the principal of which are aniline blue (§ 38), aniline black (§ 38), eosen (§ 38), chloride of gold (§ 39), pyrogallate of iron (§ 39), picro-carmine (§ 54), and several others will be found very useful.

21. *Carmine Staining.*—In the case of animal sections carmine is, as a rule, to be selected, giving, as it does, most satisfactory and beautiful results. Tissues may be stained with carmine by two different plans; in the first a strong solution is used, and the tissue subjected to its action for a very short period only, whilst in the latter, only very weak solutions are employed, the time of immersion

being considerably prolonged. The rapid method, however, is not to be recommended, for the strong carmine acts so powerfully upon the tissue as to give the various elements comprising it no time, as it were, to exercise their power of quantitative selection, but involves the whole in one uniform degree of shadeless colour. By adopting the gradual method much better results are obtained, each portion of the tissue being now at liberty to acquire its own particular shade. Amongst the various formulæ for the preparation of carmine fluid, none can be so safely followed as that devised by Dr. Lionel Beale. It runs thus:—Place ten grains of the finest carmine in a test tube, add thirty minims of strong liquor ammonia, boil, add two ounces of distilled water, and filter; then add two ounces of glycerine, and half an ounce of rectified spirit. This solution ought to be kept in a well-stoppered bottle. The best vessels in which to stain sections are small jars of white porcelain, capable of holding about two fluid ounces, and furnished with lids. They are much preferable to beakers or watch-glasses, for, owing to the white background which they afford, it is very easy to observe how the staining is proceeding. The carmine solution which we have just described, is both too strong and of too great density to be used

in its pure state. It will, therefore, require to be diluted with distilled water before use, the most serviceable degree of dilution being obtained by adding one part of stain to seven parts of water. Sections may be placed in this solution for twenty-four hours, in which time they will usually be found to have acquired a sufficient depth of colour. If, however, the tissue be unusually difficult to stain, the time of immersion may be doubled, or still further prolonged without detriment to the section.

Having prepared and filtered some of this dilute solution, say, an ounce, let us proceed to stain with it those sections which, after cutting in the microtome, we left in the basin of water (§ 11). Here we are at once met by a practical difficulty. How are the sections to be transferred from one vessel to another? This is ordinarily effected by means of a soft camel-hair pencil. It is a method, however, open to grave objection, for the sections so curl around the brush and get entangled amidst its hairs, that, notwithstanding every care, valuable sections not unfrequently become torn during transit. Every difficulty at once vanishes if we substitute for the brush a small implement which any one can readily make for himself. All that is necessary is to take a strip of German silver or copper, of the thickness of stout cardboard,

and about seven inches in length by five-eighths of an inch in breadth. The sharp angles are to be filed off and the edges carefully smoothed, whilst at a distance of five-eighths of an inch from each extremity the end must be turned up so as to form an angle of about thirty-five degrees. One end must be left plain, whilst the other, with the aid of a punch or drill, is to be

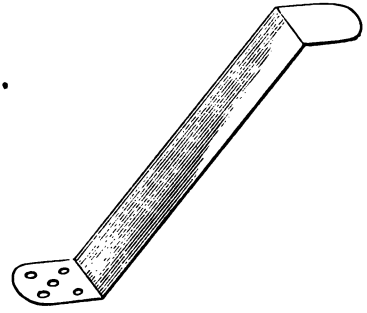

FIG. 9.—SECTION-SPOON.

pierced with five holes, each having a diameter of about the thickness of a stocking needle.*

* Dr. Klein describes a kind of "lifter," made by bending some German silver wire, but as no drawing accompanies his description it is not easy to form a clear idea as to the shape of this instrument. In the recent and philosophical work of Schäfer a lifter is figured, which consists of a wire stem, having attached to its end a spade-like blade. It will be observed that the spoon described in the text differs from this in having one end perforated, and in this consists the real value of the implement.

If we now dip the perforated end of this spoon into the water containing the sections and gently agitate it, the sections will rise from the bottom and float about. The spoon is now brought under one of them and being steadily lifted up, the water flows downwards through its apertures, and the section smoothly spreading itself out upon the spoon, may be gently lifted out of the water, and on the spoon being dipped into the staining fluid the section at once floats off. The sections having remained in the carmine fluid for about twenty-four hours, as much of the liquor as possible must be gently poured off and its place supplied by a freshly-filtered mixture of five drops of glacial acetic acid to one ounce of distilled water, when, in a few moments, the carmine will become permanently fixed in the tissue and the process of staining be complete. As the carmine fluid will not keep good for an indefinite period, only a sufficiency should be made at one time to serve for a month or six weeks. After this time the fluid, though apparently bright and clear, is very apt to deposit upon the sections, in the process of staining, numbers of bright crimson spots or stains, structureless in character and apparently of a colloidal nature. When, therefore, a fluid which has been kept for some time is used, it is as well to examine the sections

immediately after staining in order that this accident, if it has happened, may at once be detected. Should this be the case the sections must be removed to a mixture of one part of strong liquor ammonia in eight parts of distilled water, and allowed to remain in it for twenty-four hours, or until their complete decoloration has been effected. The ammoniacal liquor is then poured off and replaced by dilute acetic acid, and after a few minutes this also is to be poured off and the sections washed in several changes of clean water, when they may be re-stained either with some fresh carmine fluid or with logwood solution.

22. *Logwood Staining.*—The employment of logwood as a staining agent is now becoming very general. It acts much in the same manner as carmine, but the violet colour which it produces is by many thought to be of a more soft and agreeable character than that due to the action of carmine. A valuable and very convenient property also which it possesses is that it stains tissues very rapidly, and this without interfering with that differential kind of coloration upon which the chief value of all staining processes depends. A simple method of preparing the logwood fluid is to mix an aqueous solution of extract of logwood with a solution of alum (1 to 8) till the deep impure red colour

has become violet, and then to filter the mixture (*Frey*). This will stain sections in about half an hour. This stain, though here mentioned for the ease with which it may be made, is, as a rule, very inferior to a fluid prepared directly from hæmatoxylon, the alkaloid or active principle of logwood. As, however, it is difficult and troublesome to make the solution in this manner, it will be advisable for the student to purchase, ready prepared, such small quantity of the dye as he may require.* Small bottles may be obtained for a few pence from Mr. Martindale, 10, New Cavendish Street, London, and from repeated trials of this solution we can recommend it as producing excellent results. It is a very strong fluid, and requires to be diluted before use. The degree to which the dilution is to be carried cannot, however, be very accurately indicated, for all staining fluids of this nature possess the undesirable property of becoming decomposed by age. After the fluid has been kept for some time, a

* Should the student, however, determine to prepare this solution for himself he may proceed thus:—(1) Make a saturated solution of crystallized calcium chloride in 70 per cent. alcohol, and add alum to saturation. (2) Make also a saturated solution of alum in 70 per cent. alcohol. Add No. 1 to No. 2 in the proportion of 1 to 8. To the mixture add a few drops of a saturated solution of hæma*toxylon* in absolute alcohol.

portion of the colouring matter is thrown out of solution, and becomes deposited upon the sides and bottom of the vessel in which it is contained, hence the older the preparation, the weaker it will have become. As the time required for staining is but short, it is desirable that all the sections should begin to be submitted to its action at the same time, otherwise some will become more deeply stained than others. A good plan is to fill a small porcelain jar with filtered distilled water, and to transfer the sections into it. Whilst they are settling well down to the bottom, a mixture must be prepared of half a drachm of Martindale's solution (fresh) to one ounce of distilled water, and everything be got in readiness for its immediate filtration. The water is now very gently to be poured off the sections, and if care be exercised this may be done in such a manner as to leave them undisturbed at the bottom, after removing almost every drop of water. The diluted logwood solution must now be *immediately* filtered upon the sections, so that they may run no risk of becoming dry. In the present instance the staining may be allowed to proceed for about thirty minutes, and this will be found a convenient time for the immersion of the general run of animal sections. If the logwood fluid be not quite fresh, either a little

more of it will have to be added to the water, or the time of immersion must be prolonged, until the desired depth of colour has been produced. It is well, whilst the staining is going on, gently to shake the vessel occasionally, so that the sections may not remain in a heap at the bottom, but all be as fully as possible exposed to the action of the dye. When the staining is judged to be complete the logwood solution must be gently poured off, leaving the stained sections at the bottom of the jar, when they should be quickly covered with methylated spirit, which will fix the colour.* We shall now be able to see if the coloration obtained be perfectly satisfactory. If not deep enough, it is very easy again to submit them to the action of the dye for a few minutes longer. If, on the other hand, and as more frequently happens, the coloration should be too deep, the excess of colour may readily be removed by transferring the sections for a short time into some diluted acetic acid prepared by adding five drops of the glacial acid to an ounce of distilled water. The action of this should be carefully watched, and when the colour has been reduced to the

* Sections, after being stained with logwood, are often passed through a solution of alum to fix the colour. This, however, is unnecessary, as the action of the spirit is quite sufficient for this purpose.

desired tint the sections may be re-transferred to the methylated spirit.

23. *Double Staining.*—During the last few years many attempts have been made to stain animal sections *two colours.* This result has been endeavoured to be brought about in two different ways: (1) By first staining the section with a dye of one colour, and then re-staining it with a dye of another colour; and (2) by first mixing the different coloured dyes together, and then submitting the section to their combined influence. Various formulæ for these dyes will be found in the medical and microscopical papers, but we will here limit ourselves to the description of that one out of the several we have tried, which in our own hands yielded the best results. For this method we are indebted to the very interesting and valuable paper of Dr. J. W. Groves, which appeared in the *Journal of the Quekett Microscopical Club* for November, 1879, page 231. To carry out this process, two solutions are required—a red and a blue. To prepare the red fluid, take carmine, ½ oz.; borax, 2 dr.; distilled water, 4 oz. For the blue fluid take indigo-carmine, 2 dr.; borax, 2 dr.; distilled water, 4 oz. Mix each in a mortar and allow to stand; then pour off the supernatant fluids, to use which proceed as follows:—

1. If the sections have been hardened in chromic acid, picric acid, or a bichromate, they should be washed in water till no tinge appears.

2. Place them in alcohol for half an hour.

3. Let them remain for fifteen or twenty minutes in a mixture of the two fluids in equal parts, and after rinsing for a second in water,

4. Wash them in a saturated aqueous solution of oxalic acid, where they should remain for a rather shorter time than in the staining fluids.

5. When sufficiently bleached, wash them in water to get rid of oxalic acid, then

6. Pass them through alcohol and oil of cloves, and mount in balsam in the usual manner.

The method of double staining just described, appears to be of especial value for the treatment of pathological sections. We have some sections of scirrhosed kidney stained over a year ago by this process, in which the adventitious fibrous tissue became, and now remains, stained of a vivid and beautiful blue, whilst the remaining structures took on the rose tint of carmine. Double staining of animal tissues is yet in its infancy, and affords a wide field to those who have the necessary leisure for further developing the method, by

which valuable results in many directions are sure to be attained. We cannot leave this subject without directing the attention of all concerned in the matter to the recent valuable book of Dr. Heneage Gibbs, on *Practical Histology and Pathology*, which will be found full of practical suggestions, having reference to the various methods of compound staining.

PART IV.

On Staining Wood Sections.

24. *On Bleaching Vegetable Sections.*—Now that the practice of staining vegetable tissues, and especially vegetable sections, is so universally adopted, it has become of moment to determine by what means the previous decoloration of such objects may best be effected. That by some process or other a preliminary bleaching ought to be carried out previous to the application of staining agents will be admitted by all, but the precise process to follow so as to obtain the best results with the greatest quickness and safety does not seem so clear. The agents most commonly used for bleaching are: (1) Alcohol; (2) Solution of chloride of lime; (3) Labarraque's solution of chlorinated soda, made by decomposing lime chloride by the action of sodium carbonate. Now to each of these methods there are serious objections. Alcohol is very slow in action, and

not always certain in result. Solutions of lime chloride and chlorinated soda do bleach, it is true, but they also disintegrate and destroy, so that many delicate tissues, when subjected to the action of either of these solutions, become utterly ruined. The former solution, in addition to its direct destructive influence, has a great tendency to permit of the formation on its surface of a scum of carbonate of lime; this, sinking into the fluid, settles itself upon the sections, so that if they escape absolute destruction they are in danger of becoming coated with a brittle film, which proves equally ruinous to them. The inconveniences here mentioned led the author to discard these methods of bleaching, and to resort for this purpose to the direct action of *free chlorine*.

So far as he is aware the method is quite original, but, as "there is nothing new under the sun," it is by no means improbable that the same idea may have occurred to others. For carrying out the plan the apparatus required is simple in the extreme. All that is required is: (1) Two small wide-necked bottles —those in which chemists sell one ounce of citrate of iron and quinine are very suitable; (2) perfectly sound corks accurately fitting the bottles; (3) six or eight inches of quill glass-tubing; (4) some shellac varnish. By means

of a cork-borer or rat-tail file a hole is to be made through the centre of each cork, just large enough to grasp tightly the quill tubing. With the aid of a spirit-lamp the tube is to be bent at right angles at each end, as shown in Fig. 10. The two arms are not to be of equal length. One should be about one inch, and the other about two and a half inches. These arms must now be passed through the holes in the corks, and the corks themselves made air-tight by a liberal application of the shellac varnish.

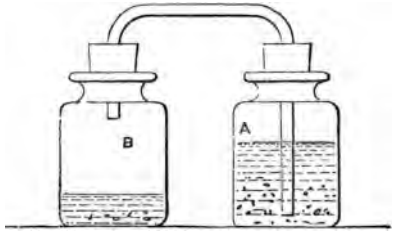

FIG. 10.—BLEACHING APPARATUS.

A notch having been cut in the edge of the cork carrying the longest arm of the glass tube, the apparatus shown in Fig. 10 is complete. To use it, proceed as follows:—About three parts fill the bottle A with filtered rain-water, and to this transfer the sections to be bleached. Into bottle B put a sufficient quantity of crystals of chlorate of potash just to cover

the bottom, and upon them pour a drachm or so of strong hydrochloric acid. Fit in the corks, taking care that the one carrying the long arm of glass tube be applied to the bottle containing the sections. Immediately, the yellow vapour of chlorine (or, strictly speaking, of euchlorine) will be observed to fill the bottle B, whence it will pass along the connecting tube into the water contained in the bottle A, and effectually and safely bleach the sections. When the water becomes supersaturated, the excess of chlorine will accumulate in the bottle above the liquid, and find an exit through the notch in the cork. As to the time required for bleaching, this will vary in accordance with the nature of the sections operated upon. As a rule, if the apparatus be set to work at night, putting it out of doors in a covered place to avoid the smell of escaping chlorine, in the morning the bleaching will generally be found to be complete; if not, further time may be allowed without any danger to the sections being incurred.

25. *On Washing the Sections.*—Decoloration having been effected nothing now remains but thoroughly to wash the sections, for it is necessary to eliminate all trace of chlorine before employing any staining agent. The usual

method of effecting this is to put the sections into a large basinful of water and repeatedly to change the latter. As this process is not only tedious but exposes the sections to considerable risk of being contaminated with dust and other extraneous matter, the author always employs in its place a system of *continuous washing*. For this purpose a small wide-necked bottle, similar to those already described, will be required. Into the side of this, half an inch or so below

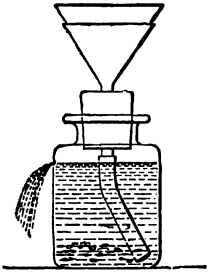

FIG. 11.—APPARATUS FOR WASHING SECTIONS.

the bottom of the cork, a small hole about an eighth of an inch in diameter must be drilled—any tinman will do this for two or three pence. A well-fitting cork being provided, this must be pierced through the centre so as to permit the stem of a small funnel to pass through it. By means of small india-rubber tubing (feeding-bottle tube) the funnel stem is to be prolonged

till it reaches the bottom of the bottle on the side *which is opposite to that side containing the perforation* (see Fig. 11). All being ready, half fill the bottle with filtered water and put the bleached sections into it. Fit in the cork carrying the funnel, and after having placed a disk of filtering paper into the funnel, put this beneath the water tap and allow a *gentle* stream to trickle into it. The water will pass to the bottom of the bottle, gradually ascend, and then pass out at the hole in the side, by which means a constant change in the water in the bottle is brought about and a system of continuous washing established. As in bleaching, so in washing, it is well to let the apparatus do its work in the night. If the tap be set running when one goes to bed, the washing will be found to have been most effectually accomplished by the time one gets up in the morning.

26. *Staining Wood Sections.*—After the sections have been bleached and thoroughly washed by the methods just described, we may proceed to stain them. This may be effected by the use of the carmine or logwood solutions; after which the sections may be mounted in balsam by the ordinary process. Better effects are, however, produced if we employ the double staining method, for the carrying out of which various kinds and combinations of dyes have

been used and recommended. It is needless to enumerate these here, since the process about to be described has yielded in our hands such satisfactory results as to render the employment of any other unnecessary. The agents used are carmine and aniline green, and to carry out the method solutions of these will be required. The solution of carmine is made by rubbing up in a mortar 15 gr. of the finest carmine with a few drops of distilled water, then adding ½ drm. of strong liquor ammoniæ and sufficient distilled water to make 7 drms. of solution. The fluid must now be exposed to the air for two days to get rid of superfluous ammonia, when 7 drms. of distilled water are to be added to it.* To prepare the green dye, take 3 grs. of aniline green, and, by means of heat, dissolve it in 2 drms. of distilled water; then filter this solution into 6 drms. of absolute alcohol.

If wood sections were, without any previous preparation, to be stained with these agents, we should find that when, in order to mount them

* Many writers recommend the carmine solution to be made with *borax* instead of ammonia. We do not like the borax solution, however, as not unfrequently sections stained by it become spotted over with an apparently crystalline deposit, which utterly ruins them. This never occurs when ammonia-carmine is employed, that is, if the solution used be *fresh* (§ 21).

in balsam, the sections were being passed through alcohol in the usual manner, a great part or the whole of the colour due to the action of the aniline green would be discharged by the alcohol. In order, therefore, to render the staining anything like permanent it is advisable to use some kind of mordant to fix the aniline stain, and for this purpose we have found tannic acid extremely useful. It is employed in the form of solution made by dissolving 1 drm. of the acid in 2 oz. of methylated spirit, and then filtering the product.

The sections to be stained, having been bleached and washed in the manner already described, must, after a short preliminary soaking in alcohol, be placed in the tannin fluid for about one minute, and thence transferred to the green dye for three minutes, upon the expiration of which time they are to be rapidly washed in distilled water, and immediately passed on into the carmine fluid, there to remain for from three to four minutes, then rinsed in dilute acetic acid (p. 80), again rapidly washed in distilled water, and finally transferred to clean methylated spirit. After remaining in this for five minutes, the alcohol must be poured off and replaced by fresh spirit, in which the sections are to remain for another five minutes, when they may be transferred to oil of cajuput.

In ten minutes or so, the cajuput oil is to be poured off and turpentine substituted for it, after soaking in which for another five minutes the sections are ready for mounting in balsam.

It must here be observed that the times given above are only approximations, and will not hold good for all cases, since the time required for the action of the stain is materially modified by the thickness of the section, and by its physical structure, and very possibly also by its chemical constitution. The exact time required in any given case can only be ascertained by actual trial; but it will be found in practice that, for the general run of wood sections, very little variation from the times indicated will be required. When an alteration, however, in this direction *is* necessary, it will be within very narrow limits, and to carry it out successfully will not tax either the ingenuity or the patience of the experimenter very severely.*

Wood sections stained by this method make beautiful preparations. Not only are certain portions of the section dyed green and crimson

* Students specially interested in the subject of double staining are referred to papers by Mr. Stiles in *Monthly Microscopical Journal*, August, 1875; Dr. Beattie, *American Journal of Microscopy*, June, 1876; Mr. Gilburt, *Journal Quekett Microscopical Club*, July, 1877; Mr. Barrett, *Science Gossip*, November, 1879; and to the *Year Book of Pharmacy* for 1878, p. 365.

respectively, but other portions acquire various intermediate tints, so that certain sections of much diversity and complexity of structure when stained by this method rival in splendour some of the diatoms.

PART V.

On Mounting Sections.

27. *Mounting Media.*—The further treatment of sections, stained by any of the preceding methods, will entirely depend upon the nature of the medium in which it is intended to mount them. There are a variety of fluids in use for this purpose, the principal being dilute alcohol (§ 37), Canada balsam (§ 32), and glycerine (§ 28). These, however, cannot be used indiscriminately, each possessing certain special properties which render it suitable for use with particular classes of objects only; thus, weak spirit having no tendency to increase the transparency of objects, can advantageously be used with such only as are already perfectly transparent. It is also more suitable for the preservation of vegetable tissues—when the retention of colour is no object—than for animal ones, since in the latter it has a tendency after a while to cause a kind of granular disintegration which

ultimately destroys much of the usefulness of the preparation. Dammar and Canada balsam, on the other hand, possess very great refractive power, so that they are of great service in mounting objects which require their transparency to be much increased. For this reason they are not well adapted to the preservation of very delicate or transparent tissues—unless previously stained—the minute details of which would become almost entirely obliterated if mounted in either of them. The chief advantage possessed by these resinous media, is that tissues mounted in them undergo no alteration even after the lapse of many years. Glycerine, in respect to its clarifying powers, occupies an intermediate position between spirit and balsam, being much more refractive than the former, infinitely less so than the latter. It is, therefore, of very great value for the preservation of such tissues as possess a medium degree of transparency, and which would become obscured if mounted in spirit, or have their outlines rendered indistinct if mounted in balsam. It is of the utmost value for mounting unstained physiological and pathological sections, which, when put up in this medium, reveal such minute details of structure as would readily have escaped observation had any other agent been employed. It may also be used with

stained sections, but in that case the sections should be of *extreme thinness*, otherwise the refractive power of the glycerine will be insufficient to render them thoroughly transparent. The great drawback to the use of glycerine is the extreme difficulty experienced in preventing its escape from beneath the cover glass, for it unfortunately possesses such great penetrating power that no cement hitherto devised can be thoroughly depended upon for withstanding its solvent action for any considerable period of time. Attention to the instructions presently to be given will, however, reduce this risk of leakage to a minimum. In the use of glycerine, Dr. Carpenter's caution must ever be borne in mind, viz., that as carbonate of lime is in course of time dissolved by glycerine, this agent ought never to be employed for the preservation of objects containing that salt.

28. *Mounting in Glycerine.*—To mount sections in glycerine we shall, in the first place, require a deep watch-glass, which is to be half filled with glycerine, diluted with an equal amount of distilled water. By means of the spoon, one or more sections may be transferred into this, either directly from the acetic acid solution in which they were placed after staining (§ 21), or, if since cutting they have been preserved in spirit, they should first

undergo a short immersion in a large vessel full of water. The watch-glass should now be covered with an inverted wine-glass, and put away for some hours, in order that the sections may become thoroughly saturated with the diluted glycerine. When this has been accomplished, a slide must be cleaned, and one of the sections, by the aid of the *unpierced* end of the spoon, be transferred to its centre.* As the kind of section with which we are now dealing is, or ought to be, of extreme thinness, no *cell* (§§ 37—45) is necessary. After tilting up one end of the slide, so as to drain off as much of the weak glycerine as possible, a drop of Price's best glycerine must, with a glass rod or pipette, be allowed to fall gently upon the section, so as to avoid the formation of air-bubbles. If any

* The appearance of a slide is vastly improved if the preparation be placed *exactly* in its centre. This may readily be done in the following manner:—Take some very finely-powdered Prussian blue and rub it up in a mortar with a little weak mucilage, so as to form a thin blue pigment. A quantity of this should be made so as always to be at hand. A slide having been cleaned, the *best surface* is to be selected, and on the reverse side, by means of a self-centring turntable, a small circle is to be drawn with a camel's-hair pencil charged with the pigment. In the centre of this ring, but on the opposite side of the slide, the section is to be placed, when it, of course, will occupy a position exactly central. When the slide comes to be finished, the blue ring may easily be removed with a wet cloth.

of these, however, should appear, they must be removed with the point of a needle set in a wooden handle,* and the slide then covered with a small bell-jar, or wine-glass. A circular cover is now to be cleaned with a soft handkerchief, and after gently blowing from it any adhering fibres of lint, etc., it will be advisable to hold the side of the glass which is to come into contact with the preparation close to the mouth, and to breathe upon it, so as to cover it with moisture. The cover, held between the thumb and forefinger of the left hand, must now be applied by its edge near to the margin of the preparation, and the surface of the cover directed in an inclined manner over it. Beneath the overhanging edge of the cover the point of the needle, held in the right hand, is now to be inserted. By gently lowering the needle the cover will come into gradual contact with the slide, driving before it a minute wave of glycerine, in which any air-bubbles that may

* A *crochet-needle* holder made of bone, and which may be bought at the smallware dealers for about sixpence, makes an admirable handle for microscopical needles. At one extremity there is a small cavity, closed with a cap, for the storage of reserve needles, whilst the other end terminates in a metal tip, provided with a crucial slit and central perforation for the reception of the needle in actual use, and so arranged that, by means of a small screw-nut, *needles* of various sizes may be firmly held in position.

have become developed are usually carried off. A very considerable degree of tact, however, is required to perform this little operation, simple as it may appear, for the retreating wave of glycerine not unfrequently floats out the section, either wholly or partially, from beneath the cover. Air-bubbles also—the *bêtes noires* of this process—are also exceedingly likely to arise. When this happens, the best plan to adopt is, by

FIG. 12.—METHOD OF APPLYING COVER.

means of the needle point, gently to raise and remove the cover, apply another drop of glycerine to the section, and cover *with a fresh piece of thin glass.* It will now be necessary to remove any superfluous glycerine which may have collected around and near the cover. The great bulk must be wiped away by means of a camel's-hair pencil, slightly wetted between the

lips, any remaining stickiness being removed with a bit of blotting paper which has been slightly damped. With a very small camel's-hair pencil, charged with solution of gelatine, a ring must be made round the margin of the cover of sufficient breadth to take in a small tract of both cover and slide. As this cement is perfectly miscible with glycerine, it readily unites with any of that fluid which may ooze from beneath the cover, and which, in the case of any of the ordinary varnishes, would act as a fatal obstacle to perfect adhesion. To make the cement, take half an ounce of Nelson's opaque gelatine, put in a small beaker, add sufficient cold water to cover it, and allow the mixture to remain until the gelatine has become thoroughly soaked. The water is now poured off, and heat applied until the gelatine becomes fluid, when three drops of creosote should be well stirred in, and the fluid mixture transferred to a small bottle to solidify. Before use, this compound must be rendered liquid by immersing the bottle containing it in a cup of warm water.

When the ring of gelatine has become quite set and dry, which will not take long, it may be painted over with a solution of bichromate of potash, made by dissolving ten grains of that salt in one ounce of distilled water. This

application of bichromate of potash should be made in the daytime, as the action of daylight upon it in conjunction with the gelatine, is to render the latter insoluble in water. When dry every trace of glycerine must be carefully removed from the cover and its neighbourhood, by gently swabbing these parts with a large camel's-hair pencil dipped in methylated spirit. After drying the slide, a ring of Bell's microscopical cement may be applied over the gelatine, and when this is dry, another coat is to be laid on. If it be desired to give to the slide a neat and tasteful appearance, it is a very easy matter, by means of the turntable, to lay on a final ring of Brunswick black or white zinc cement. Every care has now been taken to render our preparation permanent, but to make assurance doubly sure, it will be well to follow Dr. Carpenter's advice, and every year or so to lay on a thin coating of good gold size.

If square covers be employed, they may be fixed to the slide by a simple method, much in vogue in Germany. A thin wax-taper is to be lighted, and being partially inverted for a few seconds, the wax surrounding the wick will become melted. After the slide has been freed from excess of glycerine, a drop of this heated wax is allowed to fall upon each corner of the cover, and a line of the melted wax run along

the margins of the cover between these points, so as perfectly to surround it. If a good coat of white zinc cement be subsequently laid over the wax, a very durable and not unornamental line of union will have been formed.*

Mounting in Balsam.

29. *Absolute Alcohol.*—Sections cannot be mounted immediately and at once in Canada balsam. Before this can be effected there is a certain preliminary treatment to which they must be submitted. The object of this is to abstract from the tissue all its water, for if any moisture be permitted to remain in the section it will, when mounted in balsam, become obscured and surrounded by a kind of opalescent halo, due to the imperfect penetration of the

* The following is the formula for a cement to secure glycerine preparations which is recommended by the high authority of Mr. Kitton:—Take equal parts of white lead, red lead, and litharge in fine powder, grind them together with a little turpentine, until they are thoroughly incorporated, then mix with gold size to form a paint, sufficiently thin to work with a brush. This cement is to be applied in successive thin coats, each of which must be allowed to dry before another is put on. No more of this cement should be made than is required for present use, as it soon sets and becomes unworkable, but a stock of the powder may be kept ready ground in a bottle.—*Monthly Microscopical Journal*, October, 1876.

balsam into the only partially dehydrated tissue. The old-fashioned plan of dehydration was simple exposure to the air. The method now generally adopted is to bring about the same result by means of absolute alcohol. This fluid has such a strong affinity for water, that tissues submitted to its influence are rapidly and effectually deprived of any water they may contain. Absolute alcohol in small quantity may be obtained from the druggist at about sixpence per ounce. It will be necessary for the student to provide himself with a little of this agent, say, about two ounces, the method of using which will be explained by-and-by. Absolute alcohol must be kept in a bottle with a very accurately fitting stopper, in order to prevent its absorbing moisture from the air. For our purpose such a bottle, having a neck *as wide as possible*, is to be selected. After having been used a few times the alcohol will necessarily have contracted some impurities in the way of dust, etc., besides having become somewhat weaker from dilution with water abstracted from the sections; hence it will require to be passed through filter paper now and then, and a little fresh alcohol added to keep up the strength.

30. *Clove Oil.*—After having been thoroughly dehydrated the sections may, in special instances

(§ 60), be at once mounted in balsam; but as a general rule it will be found necessary, particularly in the case of animal sections, to treat them with some clarifying agent in order to remove the cloudiness and opacity which are, in part, due to their previous immersion in alcohol. For this purpose turpentine, or any of the essential oils, may be used: of these, oil of cloves is to be specially recommended. It is rather expensive, ranging from sixpence to one shilling per ounce, but as a drop or two will be sufficient for preparing each slide, only a small quantity, say, half an ounce, or an ounce, need be procured. The most convenient vessel in which to keep the oil is one of the small test bottles used by watchmakers. These bottles are provided with a glass cap to exclude dust, and the stopper is prolonged into a glass rod, which dips into the bottle. The use of this rod and the method of employing the oil will be explained shortly.

31. *Canada Balsam*, as ordinarily met with, is a thick resinous balm of great viscidity, but easily rendered perfectly fluid by the application of heat. Formerly, sections were mounted in this medium in its pure state, but owing to the annoyance which was so constantly being experienced from the tenacity with which intruding air-bubbles were held imprisoned in the viscous medium, this plan of mounting is

now rapidly falling out of use.* It is now usual to employ the balsam in a diluted condition, the chief diluents being benzol, chloroform, and turpentine. As balsam, however, often contains more or less moisture, it is desirable to drive this off before adding the diluent. A very convenient way of doing so is to expose

* Although we cannot too strongly insist upon the use of benzol-balsam wherever practicable, yet it sometimes happens in the mounting of substances of considerable thickness that, after all the benzol has evaporated, an insufficient amount of balsam is left behind to fill up the cavity between slide and cover. In such cases, therefore, it is advisable to use pure balsam, which may be done in the following manner:—The object having been previously thoroughly dehydrated by immersion in absolute alcohol, it is to be thence transferred to a little *good* turpentine or benzol, where it should remain until perfectly transparent. It is now to be placed in the centre of a slide which has been gently warmed, and a drop or two of fresh fluid balsam added, the greatest care being taken to prevent the formation of air-bubbles. Should such arise they must be touched with the point of a heated needle, which will cause them to burst and disappear. The chief difficulty of the process has yet to be encountered in the application of the cover, for it is during this procedure that the development of air-bubbles is most likely to take place. This annoyance may, however, be entirely avoided by taking the simple precaution of dipping the cover into turpentine before it is applied, when it will be found that "you can't get air-bubbles even if you try." The courtesy of Mr. J. A. Kay, late of Chatham, enables us to give our readers the benefit of this practical *wrinkle*.

some pure balsam to the heat of a cool oven for several hours, when the balsam will be found to have assumed a hard vitreous character. It should now be broken into small pieces,

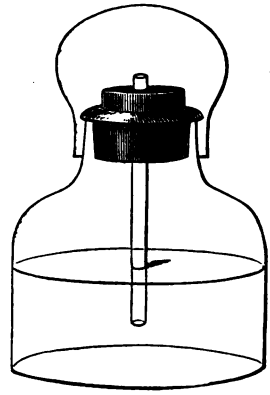

FIG. 13.—SPIRIT-LAMP ADAPTED TO CONTAIN BALSAM.

these put into a bottle, and some benzol added, which in a little while will completely dissolve the hardened balsam. More benzol is then to be added, until a solution is obtained sufficiently thin to run through filtering paper. A glass spirit-lamp must now be procured having a capacity of about two ounces, and provided with a cap. Into the wick-holder—which must be made of porcelain—of this, a hollow glass

tube is to be so fitted that its end dips into the lamp to within about a third of the bottom. The thin benzol-balsam is now to be filtered into this lamp, very fine filtering paper, through which a little benzol has first been passed, being used for the purpose. When the lamp is full it must, deprived of its cup, be put in a warm place until sufficient of the benzol has evaporated to leave behind it a fluid of the consistence of thin syrup.

32. *How to Mount in Balsam.* — We will now explain how the agents which have just been enumerated are to be employed during the process of mounting sections in Canada balsam. Though, of course, any number of sections may be proceeded with at the same time, yet, to avoid confusion in the following directions, one section only will be spoken of. This section, then, is with the perforated spoon to be transferred from the methylated spirit in which it has been soaking for some time, to the bottle of absolute alcohol, where it may remain for about an hour. Considerably less time is *actually* required; but as from constant use the spirit becomes weakened, it is as well to be on the safe side. It must now be removed to the centre of a clean glass slip, and here the *plain* end of the spoon comes into use. If this be employed for effecting the transfer it

will be found that when the section is being removed from the alcohol it will bring along with it a small pool of the spirit. A slight touch of the needle applied to the edge of the section will cause it to float off the spoon on to the slide, at the same time carrying the pool of alcohol with it, in which it will gently spread itself out upon the slide without the faintest risk of injury. The superfluous spirit is now to be drained off, and just as the section is becoming glazed and sodden-looking, *not dry*, we must, by means of the long glass stopper, apply to it a large drop of clove oil. The oil, however, should not be placed *upon* the section, but be allowed to drop upon the slide near to its margin. By gently tilting the slide the oil will gradually insinuate itself *beneath* the section, and slowly ascend through it to the surface. The slide should now be covered with a bell-jar, or wine-glass, and about two minutes allowed for the oil thoroughly to saturate the section. As much as possible of the superfluous oil must then be drained off, and the remainder removed with blotting paper. By means of the glass rod a small quantity of benzol-balsam is now taken from the spirit-lamp which contains it, and allowed gently to fall upon the section, which must then be covered with a thin glass circle, in the manner

previously described (§ 28). When the object is very fragile it is a good plan, after drawing off the clove oil, to apply the cover directly upon the section, and then to place a drop of the balsam near to the edge of the cover. This, by capillary attraction, will speedily diffuse itself beneath the cover, flowing over and surrounding the object, without in the slightest degree disturbing its position. If during the process of mounting any air-bubbles arise, we may view their development with equanimity, being well assured that as the benzol evaporates they too will quickly disappear. When the mounting is completed the slide should be roughly labelled, and placed on a warm mantel-piece for a few days to dry. Large sheets of white paper, having one surface already gummed, may be purchased from label printers or stationers at about one penny per sheet. With a gun punch scores of circular labels may be cut from such a sheet.

If a large number of slides are to be prepared at one sitting the following modification of the preceding plan will be advisable. Take a small white porcelain gallipot, with a *concave* bottom, and capable of holding about an ounce of fluid. Into this transfer some clove oil, allowing one drop for every section to be mounted. Each section is now to be removed

separately from the absolute alcohol, and all superfluous spirit being drained off by touching the edge of the section with bibulous paper, it is to be put into the oil. Here they may remain for about half an hour, when some good rectified turpentine should be filtered upon

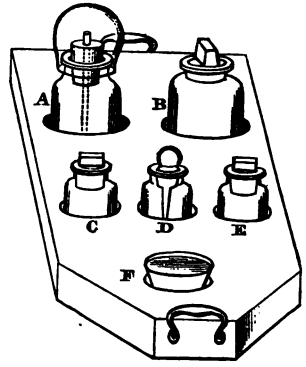

FIG. 14.—BALSAM-MOUNTING STAND.

them, until the vessel becomes about three parts filled. In the course of another half hour or so the sections may be transferred to slides, excess of turpentine removed, benzol-balsam added, and the process of mounting completed in the manner already described. This method has the merit of rapidity of execution, together with great economy in the expenditure of clove oil.

The student who is frequently engaged in mounting sections will find it very convenient and time-saving to have a small stand or frame in which all the chief requisites for mounting may be kept together. Such a frame, as shown in Fig. 14, any one may in a few minutes readily manufacture for himself out of an old cigar box. In the figure, A denotes the balsam bottle, B that for absolute alcohol, C bottle for spirits of turpentine, D for oil of cloves, E for oil of cajuput, and F the porcelain jar mentioned above. In order to exclude dust the whole stand may be covered with a bell-glass; or, what is better, each vessel may be covered with the bowl of a broken wine-glass, in which case the bottles not in actual use will at all times remain protected.

33. *Drying the Slide.*—The old-fashioned, and generally practised, plan of drying slides, mentioned at § 32, will be found the safest and most reliable that can be followed, for the heat employed is just sufficient to perform its work, but not great enough to do any damage. *Time*, however, is an important element of the process, many days being required to carry it out. Thus, where a considerable number of slides have to be dried and finished off in a limited time some other plan must be adopted.

After trying various kinds of water-bath, we gave up their use on account of the trouble involved in keeping the apparatus constantly supplied with heated water. As a substitute we devised the small copper drying-chamber, which is here figured.*

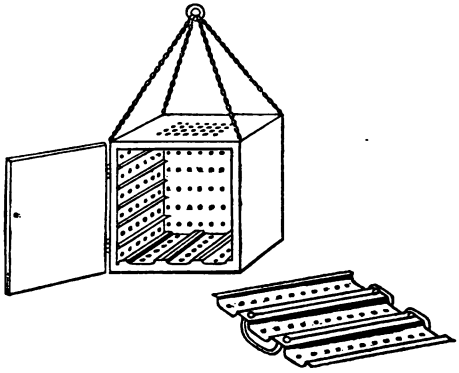

FIG. 15.—DRYING-CHAMBER FOR SLIDES.

As will be seen, the box is *double-jacketed*, the inner jacket being riddled in every direction with small holes. Along the sides of this inner jacket run on each side of the box five stout wire rods, which serve as supports for five

* In order to show more clearly that the shelves have hollowed-out bottoms and not flat ones, the comparative size of the single one figured has been purposely exaggerated.

shelves, which can be slid in and out of the box upon them. Each shelf or tray, which has the bottom also perforated, gives accommodation to three slides, and as the bottom of the box also receives three more it will be seen that the chamber will hold eighteen slides. When it is wished to use the apparatus, slides are put into it, and the box is suspended over the gas at a suitable height by means of a brass chain connected with the roof of the box. Thus, when the gas is lit in the evening, the heated air ascending, passes through the outer case of the bottom, which is perforated for that purpose, diffuses itself between the double jacket and through the interior of the chamber, and passes out through perforations in the outer casing of the roof. If desired, of course, other methods of heating might be employed; for instance, the box might be fixed on a suitable stand over the flame of a Bunsen or spirit-lamp. Slides are very quickly dried in this box, especially if every morning the superfluous balsam which has oozed from beneath the covers during the previous night's drying be removed with a hot knife, so that the remaining balsam may be directly acted upon by the heat.

34. *Drying the Slide under Pressure.*—Sometimes the section is of such a resistant and elastic nature as not to permit the cover to lie

flatly upon it. The slide must, therefore, be dried *under pressure*. Most of the contrivances in use for this purpose are worth very little. The small brass spring clips which are generally used are not to be depended upon, for when not well made—and they seldom *are* well made—they slip about from side to side in a manner most damaging to the specimen. Where pressure has to be employed, the ingenious little machine of Mr. Martin* should be used. This,

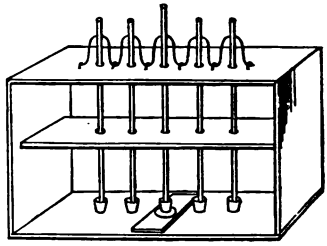

FIG. 16.—PRESSURE APPARATUS.

as will be seen from the sketch, simply consists of a piece of flat wood, having placed at a little distance above and parallel to it two similar pieces of wood, each being provided with a row of perforations. These pieces are connected to each other and to the base by two lateral uprights. Through each hole, a rod

* Martin's *Manual of Microscopic Mounting*, 1872.

of very smooth wood plays freely, and at one end terminates in a bit of very soft cork, whilst the other extremity is acted upon by an elastic cord which there passes through it. The mode of using the apparatus is so obvious, that further explanation is unnecessary. If the student will either make, or get made for him, one of these little machines, he will find it of much practical value.

35. *Finishing the Slide.*—After a sufficient length of time—which will, of course, vary according to the method of drying which has been followed—has elapsed to harden the balsam, it will be advisable to take an old penknife, and after heating the blade in the flame of a spirit-lamp, gently to run the point of it round the margin of the cover, so as to remove any excess of balsam which may have oozed from beneath it. In a day or two more any remaining balsam must be carefully scraped away with a cold knife. All remaining traces of balsam are then to be removed from around the cover by means of a rag *just moistened* with methylated spirit, or, what is better, with a mixture of equal parts of the spirit and methylated ether, after which the slide is to be thoroughly washed in cold water. The slide is now in reality finished; but in order to give it a smart appearance it is usual, with

the assistance of the turntable, to run a ring of coloured varnish round the covering-glass. It is well to let as long a time as possible elapse before the coloured cement is applied, for unless the balsam has become thoroughly hard and dry, the varnish has a great tendency to *run in* beneath the cover, a circumstance which very much impairs the elegance of the preparation. Should it be *necessary* to finish off a slide before the balsam has become quite hard, the danger of the varnish "running in" may be greatly lessened by first applying round the cover a narrow ring of thin varnish, made by dissolving shellac in methylated spirit. In order to render this ring more visible and distinct, the varnish may be coloured by stirring into it a very small quantity of any of the aniline dyes. A very useful varnish for the final coat is the white zinc cement. To prepare this, dissolve an ounce of gum dammer in an ounce of benzol. Take one drachm of oxide of zinc very finely powdered, and an equal quantity of benzol; rub them up together in a mortar, adding the benzol drop by drop, so as to form a creamy mixture perfectly free from lumps or grit. One fluid ounce of the dammar solution, previously made, must now gradually be added, the mixture being kept constantly stirred. The cement, when made,

should be strained through a piece of fine muslin, previously wetted with benzol, into a small wide-necked bottle, which, instead of having a cork or stopper, should be covered with a loose metal cap. Instead of a bottle, the varnish may be kept in one of the collapsible tubes used by artists; but though this plan is highly recommended by many, it is not without its disadvantages. If the varnish becomes thick from keeping, a few drops of benzol well stirred in will soon reduce it to a suitable consistence for use. A few words of caution here may perhaps prove useful. *Never dilute the varnish unless it is absolutely necessary to do so.* For though the addition of a little benzol to moderately thick cement makes it much more easy and pleasant to work with, yet the addition cannot always be made with safety; for if an excess of the solvent should be added, upon the evaporation of this there will remain behind, an insufficient amount of gum dammar to retain the colouring matter employed; hence, on drying, the varnish will lose its lustre, present a granular or floury appearance, and ultimately disintegrate and crumble away.

36. *Care of the Hands.*—As the practical microscopist is constantly working with viscid and discolouring materials, he will find no

small difficulty in keeping his hands perfectly clean. Whatever care he may exercise, smears of balsam, varnish, etc., *will* get upon his fingers. The usual method of removing these is the application of some one of their solvents. Turpentine, however, the agent most commonly used for this purpose, is both dirty in use and disagreeable in smell, whilst benzol and ether are too costly. Mr. Archer, of Liverpool, has recently patented a small slab of pumice stone, having one of its surfaces chased into small quadrangular facets or dice. In this little article, which has been named *the patent chequered pumice tablet*, the microscopist will find a true friend. If, whilst washing the hands he will use this little scrubber, keeping its facetted surface well smeared with soap, he will find all smears and stains vanish like magic under its attriting action. These tablets, the price of which is quite nominal—threepence to sixpence each—may be procured from Messrs. Jackson, wholesale chemists, Liverpool.

PART VI.

On Special Methods of Preparation.

Having in the preceding pages entered at some length into the general subject of section-cutting, it remains for us now to consider those special methods of preparation which the peculiarities of certain structures demand. In order to keep the bulk, and consequently the price, of this manualette within due bounds, we shall, without further preface, proceed to the description of these methods, in doing which every endeavour will be made to employ such brevity of expression as may be consistent with perfect clearness of meaning. As the most convenient plan, the objects here treated of will be arranged in alphabetical succession.

37. *Bone.*—Both transverse and longitudinal sections should be prepared, the former being the prettier and most interesting. After prolonged maceration in water, all fat, etc., must be removed and the bone dried, when as thin a

slice as possible is to be cut off in the desired direction by means of a very fine saw. If the section so obtained be placed upon a piece of smooth cork it may, with the aid of a fine file and the exercise of care, be further reduced in thickness. It is then to be laid upon a hone moistened with water, and being pressed gently and *evenly* down upon it with the tip of the finger—protected, if necessary, by a bit of cork or gutta-percha—it must be rubbed upon the stone until the desired degree of thinness has been attained. Finally, in order to remove scratches and to polish the section, it should be rubbed upon a dry hone of very fine texture, or upon a strop charged with putty-powder. After careful washing in several waters the section must be allowed thoroughly to dry, when it may be mounted by the *dry method* in the following manner:—A ring of gold size must, by means of the turntable, be drawn in the centre of a slide, and the slide put away in a warm place for several days (the longer the better), in order that the ring may become perfectly dry and hard. When this has been accomplished the section is to be put in the centre of the ring, and a covering glass of the requisite size having been cleaned, this must have a *thin* ring of gold size applied round its margin. The cover is now placed in position

and gently pressed down, the pressure apparatus being employed, if necessary, to prevent it from moving. In about twenty-four hours another layer of the varnish should be applied, and the slide afterwards finished in the manner already described. The above method is also applicable to the preparation of sections of *teeth*, and also of *fruit-stones* and other hard bodies, which are incapable of being rendered soft enough for cutting. Sections of bone may also be mounted in Canada balsam, but this agent, by penetrating the canaliculi and lacunæ, renders them invisible. To prevent this, prepare a solution of gelatine, and, with a camel's-hair pencil, apply a *very thin* coating of this solution over each surface of the section; allow two hours for this to dry, then mount in balsam.*

As the process just described, however, is both troublesome and tedious, it is much better for ordinary purposes to have recourse to the *decalcifying method*, by which means sections in every way suitable for the examination of the essential structure of bone may be obtained with ease. To carry out this plan a piece of fresh bone should be cut into small pieces and placed in a solution made by dissolving 15 gr. of pure chromic acid in 7 oz. of distilled water, to which 30 min. of nitric

* *Traité du Microscope*, par Ch. Robin, 1871, pp. 345, 346.

acid are afterwards to be added. Here they should remain for three or four weeks, or until the bone has become sufficiently soft to cut easily, the fluid being repeatedly changed during the process. From this solution they must be transferred to methylated spirit for a few days, when a piece may be selected, imbedded in paraffin, and cut in the microtome. Some of the sections should be mounted, unstained, in spirit. For this purpose a cell of gold size, as above described, must first be prepared and filled *full* of a mixture of spirit of wine one part, and distilled water three parts. Into this the section (after having previously been soaked for a time in some of the preservative solution) must be carefully placed and the cover applied, the same precautions for the exclusion of air-bubbles being taken which were recommended when speaking of mounting in glycerine. When the cover is in position a ring of gold size must be laid on, repeated when dry, and the slide afterwards finished in the ordinary manner. It will be advisable to stain some of the sections with carmine, or picro-carmine (§ 54), and mount them in glycerine. *Teeth* may also be treated by the decalcifying method, but in this case it must be remembered that the enamel will dissolve away.

38. *Brain.*—The best hardening fluid is that recommended by Rutherford, and is made by dissolving 15 gr. of pure chromic acid and 31 gr. of crystallized bichromate of potash in 43 oz. of distilled water. Small pieces of brain, which have previously been immersed for twenty-four hours in rectified spirit, should be placed in about a pint of this solution, where they may remain for five or six weeks, the fluid being repeatedly changed during the process. If by this time they are not sufficiently hard, the induration must be completed in alcohol. Sections are easily cut in the microtome by the paraffin method. They may advantageously be stained by Frey's *aniline blue solution*, made by dissolving one grain and a half of aniline blue in ten ounces of distilled water, and adding one drachm of rectified spirit. As this stain acts very rapidly, two or three minutes immersion will generally be found long enough. The sections must then be mounted in balsam. Sections may also be stained with *eosen*, as recommended by Prof. Dreschfeld.* The solution, which will keep good for any length of time, is made by simply dissolving one part of eosen in 1,000 parts of distilled water. Sections may lie immersed in this solution for

* *Journal of Anatomy*, vol. xi. part 2.

from sixty to ninety seconds, then transferred for a short time to diluted acetic acid, and finally mounted in balsam or glycerine.

A very excellent and easily carried-out plan for investigating the structure of *perfectly fresh* brain has been suggested by Dr. Bevan Lewis. The chief value of this method is the extraordinary clearness with which *isolated* brain structures, and particularly the cells, are brought into view. For the study of *structural* relationship ordinary sections are to be preferred. The details of the process are as follow:— After being deprived of its membranes, a piece of brain of convenient size is to be removed, and vertical sections, including the cortex, as thin as possible, cut with a broad razor, the upper surface of which should be deeply concave. Both knife and tissue are kept deluged with spirit during the cutting. A good section having been obtained, it is to be placed on a glass slip, and a few large drops of Müller's fluid* let fall upon it from a glass pipette. After some seconds, when the section has become thoroughly soaked with the fluid, a large covering glass must be applied and pressed down steadily and gently until the

* Müller's fluid is made by dissolving 25 grammes of bichromate of potash and 10 grammes of sulphate of soda in 1,000 cubic centimetres of water.

section becomes flattened out into a thin, almost invisible film. All superfluous fluid is removed by rapidly rinsing in water, and the slide transferred to a flat porcelain dish containing methylated spirit. In about forty seconds the slide is removed, one edge of the cover steadied with the finger, and the blade of a penknife gradually inserted beneath the opposite edge and the cover removed, when the section or film will be found either loosely floating on the slide, or closely adherent to the cover. The slide or cover to which the film is attached, must now be slightly inclined, and a gentle stream of water from a large camel's-hair brush allowed to run over it. A large drop of a one per cent. solution of aniline black is now let fall upon the section. When the requisite colour has been acquired—a matter learned by experience only—the slide is very gently lowered into a vessel of water, and allowed to rest upon the flat bottom. In a short time the film will be observed to become of a deeper hue, and the staining perfectly uniform. The slide must then be carefully removed from the water, all fluid drained off, and the preparation be placed beneath a bell-glass until it is perfectly dry, when a few drops of chloroform are to be let fall upon the film. After a few seconds a drop of chloroform or benzol-balsam

is to be added, a cover applied, and the slide finished in the usual manner.*

39. *Cartilage.*—The method to be employed in the preparation of cartilage will entirely depend upon the nature of the staining agents, to the action of which the sections are to be submitted. Thus, if the elegant *gold method* is to be followed, it is necessary that the cartilage should be perfectly fresh; whilst if any of the other staining agents are to be employed the tissue may have been previously preserved in alcohol. An excellent object on which to demonstrate the gold process is to be found in the articular cartilage of bone. It is a very easy matter to obtain from the butcher's the foot of a sheep which has just been killed. The joint is to be opened and the bones dissociated, when they will be seen to have their extremities coated with a white glistening membrane—this is the *articular cartilage.* Exceedingly thin slices must be at once cut from it, and as only small sections are required, a sharp razor may be used for the purpose, the blade being either dry or simply wetted with distilled water. The sections as cut are transferred to a small quantity of a half per cent. solution of chloride of gold in a watch-glass. Chloride of gold may be purchased in small glass tubes

* *Monthly Microscopical Journal*, September, 1876.

hermetically sealed, each tube containing fifteen grains, and costing about two shillings. If, however, the student requires only a small quantity of the staining fluid he need not be even at this small expense, for, as photographers for the requirements of their art always keep on hand a standard solution of chloride of gold of the strength of one per cent., a little of this may readily be obtained and diluted with distilled water to the required degree. After the sections have been exposed to the action of the staining fluid for about ten minutes they may be transferred to a small beaker of distilled water and exposed to diffused daylight for about twenty-four hours, when they must be mounted in glycerine.

Sections of cartilage may also be examined without being stained, in which case the field of the microscope should be only very feebly illuminated; or carmine staining may be resorted to. These sections show well in glycerine, or if the staining be made very deep even Canada balsam may be employed, and with fair results.

Microscopists are indebted to Dr. Frances Elizabeth Hoggan for the description of a new method of staining which we have found especially suited to the treatment of cartilage. The agent employed is *iron*, and the process, which is

very simple, is as follows:*—Two fluids are necessary. (1) Tincture of steel; and (2) a two per cent. solution of pyrogallic acid in alcohol. A little of the former is to be poured into a watch-glass, and into this the sections, after having been previously steeped in alcohol for a few minutes, are to be placed. In about two minutes the iron solution is to be poured away and replaced by solution No. 2. In the course of a minute or two the desired depth of colour will have been produced, when the sections are to be removed, washed in distilled water, and mounted in glycerine. The results obtained by this process are very beautiful, the colour produced being a fine neutral tint of delightful softness. The process also answers admirably in the case of morbid tissues, and we have now in our possession some sections of ulcerated cartilage tinged by the iron method, in which the minute changes resulting from the ulcerative degeneration are brought out with wonderful distinctness.

We may also stain cartilage sections first with carmine, and, then, after washing in water slightly acidulated with hydrochloric acid, with a very dilute aqueous solution of aniline blue.

* For the purpose of hardening tissues which are to be stained by Dr. Hoggan's method, alcohol should be employed, the use either of chromic acid or bichromate of *potash being* inadmissible.

Such double stained sections show the cellular elements red, the elastic blue.*

As the structure of cartilage differs according to its purpose and situation, the student will find his time profitably employed in a careful examination of the following forms: (*a*) *hyaline*, articular and costal; (*β*) *yellow fibro-cartilage*, epiglottis and external ear; (*γ*) *cellular*, ear of mouse. Sections of the *intervertebral ligaments* should also be made, in which the different kinds of cartilage may be examined side by side with each other.

40. *Coffee Berry* affords sections of great beauty. The *unroasted* berry should be soaked for some hours in cold water until sufficiently soft,† then imbedded in paraffin and cut in the microtome, the section being made in the direction of the long axis of the berry. Only a few perfect sections can be obtained from one berry, for at the *hilum* the seed-covering turns in and penetrates the substance of the berry to such a depth, that before many sections have been made, the reflected covering will be cut down upon. When this has been reached a

* *Leitfaden zur Anfertigung mikroscopischer Dauerpräparate.* Von Otto Bachmann. München, 1879. Page 142.

† Seeds should not be allowed to remain *too long* in water, especially in warm weather, otherwise they will begin to germinate, in which case their internal structure becomes entirely changed.

circular ribbon representing the covering, will—unless in exceptional cases—fall from the centre of the section and mar its beauty. Sections of coffee berry may be mounted in glycerine, or stained rather strongly with carmine and mounted in balsam. The same method of treatment applies to all other hard berries or seeds.

41. *Chicory.*—When the student has secured his slide of coffee, he will do well to prepare a longitudinal section of unroasted chicory-root for comparison. He will then observe the striking difference between the two substances, and see how easy it is to distinguish one from the other. Thus, in the case of coffee, the cells will be noticed to be small and more or less *angular*, whilst those proper to chicory present a round or oval outline. In addition to the cells, the section of chicory will also reveal the presence of *dotted ducts*, which are entirely absent from coffee. To make the comparison complete, a coffee berry should be soaked in water until the skin or seed-coat becomes loosened. A portion of this is then, with the point of a knife, to be removed to a slide and examined in glycerine, when it will be found to consist of a hyaline membrane in which are imbedded small rod-like bodies of elliptical shape, and having their long axes *running* in the same direction. These rods

cannot be mistaken for any of the *ducts* met with in chicory.

42. *Fat.*—Adipose tissue may be hardened in alcohol, cut in paraffin, and mounted in glycerine. If the tissue has been injected, the sections may be mounted in balsam, and are then very pretty objects, showing the capillary network encircling the cells. In sections of fat which have been mounted for some length of time in glycerine, it will frequently be noticed that the fatty acids become precipitated and crystallize within the cells, an accident which greatly improves the slide.

43. *Hair.*—Longitudinal sections are readily made by splitting the hair with a sharp razor. It is more difficult to cut the hair transversely. This, however, may easily be done in the following manner. The hairs having previously been well soaked in methylated ether to remove all fatty matters, a sufficient number of them must be selected to form a bundle about the thickness of a crow quill. This bundle, after being tied at each extremity with a bit of thread, is to be immersed for several hours in strong mucilage to which a few drops of glycerine have been added. On removal, the bundle must be suspended, by means of a thread attached to one end of it, in a warm place until sufficiently hard, when it is to be imbedded and cut in

paraffin. Each section, as cut, is to be floated off the knife into methylated spirit. From this it is with the aid of the spoon to be transferred to a slide, the spirit tilted off, a drop of absolute alcohol added, when, after a minute or two, this also is to be drained off, the section treated with clove oil, and the mounting completed in the manner described in previous pages.

44. *Horn* varies very much in consistence, in some instances having a cartilaginous character, whilst in others it is almost bony. In the latter case sections will have to be ground down in the manner described when speaking of bone. Where the texture is less dense recourse may be had to prolonged steeping in hot or boiling water; in some cases it will be necessary to continue the immersion for several hours. When sufficiently soft, the piece of horn may, by means of bits of soft wood, be firmly wedged into the tube of the microtome, or secured in Swift's adjustable vice (p. 66), and sections cut with a razor, or, what is better, with a broad and very sharp chisel. The sections are to be put between glass slips, held together by American clips, or pegs, and put away for two or three days in order to become thoroughly dry. After well soaking in good turpentine or benzol, they must be transferred to slides, the superfluous turpentine drained off,

and the sections then mounted in benzol-balsam. Sections of *horn* should, of course, be cut in different directions; but for examination with the polariscope those cut transversely yield by far the most magnificent results. *Hoofs*, *whale-bone*, and allied structures should also be treated by the above method.

45. *Intestine.*—The method to be pursued with *sections* has already been described. The ileum, however, is a very pretty object when a portion of it is so mounted as to show the *villi erect*. To do this, it is necessary to cement to the slide, by marine glue, a glass cell of sufficient depth. This should have been prepared some time beforehand, so that the cement may be perfectly dry and hard. The cell is now to be filled with turpentine, and the piece of ileum (having been previously passed through methylated spirit and absolute alcohol into turpentine) is gently placed into it, having the villi uppermost; pour some pure and rather fluid balsam on the object at one end, and gradually incline the slide, so as to allow the turpentine to flow out at the opposite side of the cell, till it is full of balsam. Then take a clean cover, and having placed upon it a small streak of balsam from one end to the other, allow it gradually to fall upon the cell, so as to avoid the formation of air-bubbles (*Ralf*),

and finish the slide in the usual manner. Or, the intestine may be dried, and mounted dry in a cell with a blackened bottom, for examination as an opaque object.

46. *Liver.*—Small pieces of liver may be very successfully hardened by immersion in alcohol, beginning with weak spirit and ending with absolute alcohol. Sections from liver, the portal and hepatic veins of which have been filled with different coloured injections, make capital *show* slides, the boundary of each lobule being accurately mapped out by the one colour, whilst from its centre the other colour is seen to radiate to the circumference.

47. *Lung* must be prepared in chromic acid. For the cutting of sections the freezing microtome is of especial value, and should, therefore, if possible, be used. If, however, the student be not provided with this instrument, he may either employ the etherized paraffin method (§ 17), or proceed as follows:—A small piece of lung, previously deprived of all spirit by steeping for some time in water, is to be immersed till thoroughly saturated in solution of gum (§ 14). A small mould of bibulous paper, only just large enough to conveniently receive the piece of tissue, having been prepared and filled with the mucilage, the specimen must be transferred to it. The

mould with its contents is now to be placed in a saucer, into which a mixture of about six parts of methylated spirit and one part of water is to be poured (*Schäfer*), until the fluid reaches to within about a third of the top of the mould. In the course of several hours the surface of the mucilage will begin to whiten and solidify. As soon as this occurs more dilute spirit must be poured into the saucer until the mould is completely submerged. In a day or two the gum will be found to have acquired a suitable consistence for cutting, when it must be removed from the spirit, the paper mould peeled off, and the mass imbedded and cut in paraffin, the sections being afterwards treated as if they had been obtained by the freezing method (§ 14). If the solidification of the gum should proceed too slowly, a few drops of pure spirit may be occasionally added to the contents of the saucer. If, on the other hand, the gum should become over-hard, it will be necessary to let fall into the saucer a few drops of water, and to repeat this until the required consistence be obtained.

48. *Muscle.*—Harden in chromic acid, and cut in paraffin. Transverse sections may be made to show the shape of the fibrils. Longitudinal sections will only be required in the case of injected tissues, when such sections will be found very elegant, showing, as they do, the

elongated meshes of capillaries running between and around the muscular fasciculi. Mount in glycerine or balsam. To see the transverse striæ, characteristic of voluntary muscle, a very good plan is to take a bit of pork—cooked or fresh—and by means of needles, to tease it out into the finest possible shreds. If these be examined in water or glycerine, the markings will be shown very perfectly.

Whilst speaking of muscular tissue it may not perhaps be altogether out of place to refer briefly to that most pernicious parasite, the *Trichina spiralis*, which, in the muscles of both man and beast, builds for itself a nest, to the discomfort, and, in many cases, to the imminent danger of its *host*. The flesh of pigs and cows, and especially that of the former, is not unfrequently found to be largely infested with the Trichina spiralis in an encysted condition. The flesh of these animals thus affected being consumed by man, the encysted trichinæ become liberated from their envelopes, and in a few days, whilst still in the alimentary canal, arrive at a state of sexual maturity, and speedily produce an immense progeny of embryos. These, either by passing along the blood-vessels, or lymph-canals, or by boring through the intestinal walls, ultimately find their way into the muscular tissues. After wandering about here

for some time they penetrate the muscular sheath, and between the ultimate muscular fibrilla, roll themselves up in a spiral form and become encapsuled. In course of time the capsule hardens, and finally becomes converted into a calcareous envelope. In this condition, so far as the *host* is concerned, the parasite becomes harmless. Before, however, this satisfactory state of affairs is attained, the trichinæ, by their *wanderings*, not unfrequently produce in the unfortunate creature who is their entertainer a peculiar febrile condition, always attended by extreme suffering, and, in many cases, terminating most disastrously. Since such serious consequences may follow from the consumption of flesh infested with trichinæ, it is no little satisfaction to know that, in the case of any suspected meat, we possess a simple and easy method of demonstrating the presence or absence of the parasite in it. All that is necessary to do, is to make a thin longitudinal section of the suspected muscle, place it upon a slide, add a drop of water, apply the cover, and press it firmly down. If the parasite be present, we may find it—(1) *free*, either amongst the muscular fibres, or floating in the fluid surrounding them. Its form may be various, either spiral, S-shaped, or more or less straight. (2) Enclosed in a *transparent cyst*, in which the

worm will be found coiled up in a spiral; or, (3) the capsule may be *calcareous and opaque*, in which case nothing will be seen but the oval capsule. The addition, however, of a few drops of dilute acetic or hydrochloric acid, will render the capsule transparent and reveal within it the spirally coiled parasite. A low power of from × 30 to × 60 will be sufficient for purposes of identification, but to examine the internal structure of the trichina × 200 or more will be required.

We have every reason to believe that the presence of trichinæ in the human body is much more frequent than is generally supposed. In the Manchester School of Medicine we saw some time ago a subject, the muscles of which were crowded with wandering trichinæ, whilst quite recently a friend of ours removed, in the dissecting room at Leeds, a portion of the sterno-mastoid muscle from a subject there, which, on examination, was proved to be literally swarming with trichinæ in an encysted condition. Since the public safety is so deeply concerned in this matter, it is a wonder to us that the same appliances which are in use on the Continent for detecting infected meat are not put into the hands of our sanitary inspectors. A microscope, so cheap in cost and so very easy of employment that it might be put into the hands of

every meat inspector, has been devised for this special purpose by Dr. Hager, and called by him *the patent compressor microscope.* The accompanying sketch scarcely requires any explanation. It will be seen that the microscope is provided with a spring acting upon a ring which rests upon the stage plate.

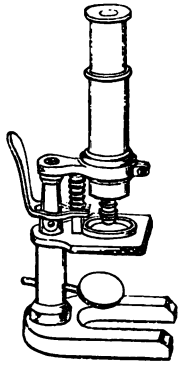

FIG. 17.—TRICHINA MICROSCOPE.

In using the microscope, all that is required to be done is to put a morsel of the suspected muscle on the slide, add a drop of water, cover with another slide, raise the ring by pressing a lever behind the upright of the stand, and put the slides upon the stage; when the lever is released, the ring descends and presses the

slides together, so that a very thin film of muscle remains for examination, in which the presence of trichinæ, should such be there, is readily detected.*

49. *Orange Peel*, common object though it be, is not to be despised by the microscopist. Transverse sections must be prepared by the gum method. These sections are not to be subjected to the action of alcohol—as this would destroy the colour—but after drying between glass slips, they must be soaked in turpentine and mounted in balsam. We shall then have a good view of the large globular glands whose office it is to secrete that essential oil upon which the odour of the orange depends. Sections may also be bleached, and stained with carmine and aniline green (§ 26).

50. *Ovary* may be prepared in the same manner as liver. Sections, which are to be cut in paraffin, may be stained with carmine and mounted in glycerine or balsam. Apart from all scientific value, we know of no slide for the microscope, which, even as a mere object of show, surpasses in beauty a well-prepared section of *injected* ovary, exhibiting the wondrous Graafian vesicles, surrounded by their meandering capillaries.

* *Das Mikroskop und seine Anwendung.* Von Dr. Herrmann Hager. Berlin, 1879. Page 41.

51. *Porcupine Quill.*—Soften in hot water, cut in paraffin, and mount in balsam for the polariscope. Sections, which must be cut very thin, should be taken from the tapering extremity of the quill in order that the whole section may come into view when examined with a moderate power.

52. *Potato.*—From the large amount of water which it contains thin sections cannot be cut from the potato in its natural state. It must, therefore, be partially desiccated, either by immersion in methylated spirit for a few days or by exposure to the air. Sections may then readily be obtained by imbedding and cutting in paraffin. Such sections mounted in balsam are very beautiful, the starch being seen *in situ*, whilst, if polarized light be employed, each granule gives out its characteristic black cross.

53. *Rush* is to be prepared and cut as orange peel. Transverse sections of this "weed" furnish slides of the most exquisite beauty.

54. *Skin.*—To prepare skin for section a piece is to be selected, which, after having been boiled for a few seconds in vinegar, must be stretched out on a bit of flat wood, and being maintained in position by pins, allowed to dry. Then imbed in paraffin, and cut *exceedingly thin* sections. These may be stained with carmine, but more beautiful results are obtained if picro-

carmine be employed. Sections of skin when stained by this agent are much increased both in beauty and instructiveness; for, the several constituents of the tissue becoming tinged with different colours are readily distinguishable from each other, whilst the contrast of colouring forms a pleasing picture to the eye. The method of preparing picro-carmine is very simple, though it sometimes yields a solution not altogether satisfactory. The best formula with which we are acquainted is that given by Rutherford, in his book already mentioned, and if due care be taken in following it out failure will generally be avoided:—"Take 100 c.c. of a saturated solution of picric acid. Prepare an ammoniacal solution of carmine, by dissolving 1 gramme in a few c.c. of water, with the aid of excess of ammonia and heat. Boil the picric acid solution on a sand-bath, and when boiling, add the carmine solution. Evaporate the mixture to dryness. Dissolve the residue in 100 c.c. of water, and filter. A clear solution ought to be obtained; if not, add some more ammonia, evaporate, and dissolve as before." Sections may be exposed to the action of this fluid for a period varying from fifteen to thirty minutes, then rapidly washed in water, and mounted in glycerine. *They* may also be mounted in balsam, care

being taken in that case to shorten as much as possible the period of their immersion in alcohol, so that no risk may be run of the picric acid stain being dissolved out.

If it is intended to study the structure of skin with anything like thoroughness, portions must, of course, be examined from different localities, in order that its several varieties and peculiarities may be observed. Thus, the *sudoriferous*, or, sweat glands, may be found in the sole of the foot, whilst the *sebaceous* glands are to be sought in the skin of the nose. The *papillæ* are well represented at the tips of the fingers,* whilst the structure of the shaft of the *hair*, together with that of the follicle within which its root is enclosed, as also the muscles by which it is moved, are to be studied in sections of skin from the scalp or other suitable locality.

Vertical sections of skin form very pleasing objects for the polariscope. For this purpose almost all kinds of skin may be employed, that of the *crocodile* being particularly interesting.

* It is well, in connection with these papillæ, to bear in mind a fact pointed out by *Frey*, namely, that the tips of the fingers frequently become, *post-mortem*, the seat of extensive natural injections. Hence, in sections from this region, we frequently obtain good views of distended capillaries without having been at the trouble of previously injecting them.—*Microscopical Technology*.

The sections should be cut very thin, and mounted, unstained, in balsam.

55. *Spinal Cord.*—The spinal cord—say, of a cat or dog, or, if procurable, of man—having been cut into pieces about half an inch in length, may be hardened in the usual chromic acid fluid. As it is peculiarly liable to over-harden and become uselessly brittle, the process must be carefully watched. Its further treatment is the same as that of brain. The sections may be stained very satisfactorily by the *ink process*, for communicating details of which we are indebted to the kindness of Dr. Paul, of Liverpool. The agent usually employed is Stephens' blue-black ink, which for this purpose must be quite fresh. As in the case of carmine, two methods of staining may be adopted, either rapid, by using concentrated solutions, or more prolonged, according to the degree to which the ink has been diluted. For the reasons previously given (§ 21) slow methods of staining are always to be preferred, as yielding the most beautiful results; yet for the purposes of preliminary investigation, it is often convenient to have recourse to the quick process. To carry out the latter plan, an ink solution of the strength 1 in 5 to 10 parts of water is to be freshly prepared, and the sections exposed to its action for a few minutes. For

gradual staining, the dilution is to be carried to 1 in 30, or 1 in 50, and the time of immersion prolonged to several hours, the sections being occasionally examined during the staining, so that they may be removed just as they have acquired the desired tint. When a satisfactory coloration has been obtained the preparations should be mounted in dammar or balsam. One advantage of this method of staining is that definition is almost as good by artificial light as by day.

56. *Sponge* may readily be cut after being tightly compressed between two bits of cork, or its interstices may previously be filled up by immersion in melted paraffin or mucilage, and sections cut in the usual manner.

57. *Stomach* requires no special method of hardening (chromic acid). Sections, when practicable, should always be cut in the freezing microtome. In default of this proceed in the manner as directed for lung, or use the etherized paraffin method. Both vertical and horizontal sections will, of course, be required. If the preparation has been injected, the latter are particularly beautiful. Stain with carmine or with aniline blue (§ 38), and mount, if for very close study, in glycerine; if injected and for a show slide, use Canada balsam.

58. *Tongue.*—Harden in chromic acid, imbed,

and cut in paraffin. As, however, the paraffin is apt to get entangled amongst the papillæ, whence it is afterwards with difficulty dislodged, it will be as well before imbedding to soak the tongue in weak mucilage for a few minutes, and afterwards immerse in methylated spirit till the gum becomes hardened, so that the delicate papillæ may thus be protected from the paraffin by a surface coating of gum. The best staining agent is picro-carmine. Sections of *cat's* tongue, near the root, when thus stained, furnish splendid objects. Sections should also be made of the *taste bulbs* found on the tongues of rabbits. These are small oval prominences situated one on each side of the upper surface of the tongue, near its root. They should be snipped off with scissors, and vertical sections made in the direction of their long axes. Stain with carmine or picro-carmine, and mount in glycerine or balsam.

59. *Vegetable Ivory*, after prolonged soaking in cold water, may readily be cut in the microtome. The sections should be mounted unstained in balsam, and though not usually regarded as polariscopic objects, nevertheless when examined with the *selenite* yield very good colours.

60. *Wood.*—Shavings of extreme thinness *may be* cut from large pieces or blocks of timber

by means of a very sharp plane. In this way very good sections may be procured of most of the common woods, as oak, mahogany, *glandular wood* of pine, etc. Where, however, the material to be operated upon takes the form of stems, roots, etc., of no great thickness, they should, after having been reduced to a suitable consistence (§ 2), be imbedded in paraffin and cut in the microtome. Before imbedding, it must not be forgotten to immerse the wood to be cut in weak gum-water, this precaution being of great importance, especially in the case of stems, etc., the bark of which is at all rough and sinuous. If the sections are to be mounted *unstained*, they are usually put up in weak spirit (§ 37). A very general method also of dealing with this class of objects is to mount them dry (§ 37). This plan, however, cannot be recommended, for however thin the sections may be, the outlines, when this process is adopted, always present a disagreeable, black, or blurred appearance. To avoid this, we may have recourse to Canada balsam, but the ordinary method of employing it must be slightly modified, a drop of chloroform being substituted for the clove oil (§ 32), otherwise this latter agent would cause the section to become so transparent as to render minute details of structure difficult to recognize.

For the methods of *staining* wood sections, the reader is referred to § 26.

The preceding list by no means represents all the objects, sections of which will be found interesting to the microscopical student. Such was not its purpose; had it been so, the enumeration might have been prolonged almost indefinitely. The end in view was to bring under the notice of the reader only those substances the preparation or cutting of which is accompanied by difficulty, and even of this class, the space at our disposal has been so limited, that we have been unwillingly compelled to pass over many, and to dwell only on such as possess a typical character.

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